1996

Liquid chromatographic retention studies using polystyrene-divinylbenzene stationary phases in reversed-phase and normal-phase eluents

Thomas Kent Chambers
Iowa State University

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

Liquid chromatographic retention studies using polystyrene-divinylbenzene stationary phases in reversed-phase and normal-phase eluents

by

Thomas Kent Chambers

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department: Chemistry

Major: Analytical Chemistry

Major Professor: James S. Fritz

Iowa State University

Ames, Iowa

1996

Graduate College
Iowa State University

This is to certify that the Doctoral dissertation of

Thomas Kent Chambers

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

DEDICATION

To James K. Chambers, my father, the biggest man I know.

To Max R. Chambers, his father, for making a lasting impression.

To Sarah Chambers, my wife, for her faithful love and support.

To Jesus Christ, for His sacrifice and my redemption.

TABLE OF CONTENTS

GENERAL INTRODUCTION

Dissertation Organization

This dissertation comprises four primary segments, each reported separately in papers which have been, or will be submitted to refereed journals in the field of analytical chemistry. The separate sections follow the general format used for publication of the individual papers. Abstracts and reference sections are provided separately for each paper. A general introduction is presented first, including a review of pertinent literature. In order to bring continuity to the entire work, general conclusions are advanced in a section following the four main chapters.

Retention Studies in High-Performance Liquid Chromatography

The term "high-performance liquid chromatography" (HPLC) has been adopted since about 1970 to denote a chemical separation technique which uses a high-pressure pump to force a liquid (mobile phase) through a column packed with a solid material (stationary phase) [1,2]. The technique was developed as an improvement over open-column chromatographic separations, which were less precise and required long analysis times. One advantage of HPLC over earlier methods lies in the greater separation efficiency afforded by the high surface area and small particle diameter of the stationary phases. Low-performance open-bed columns relied on gravity for mobile phase flow and could not accommodate small-diameter particles due to their restrictive effect on flow rate. Other advantages of HPLC over traditional open-bed liquid chromatography are precise sample injection, automation, on-line detection, and ability use columns repeatedly and thus spread the cost of the column over

several analyses [2]. High-performance liquid chromatography also offers higher peak resolution than classical open-bed liquid chromatography.

Beginning with the earliest liquid chromatographic separations through the present, it has become important to understand the separation process in order to maximize efficiency and selectivity. Since the separation of two analytes in liquid chromatography depends on their relative migration through the column, it is important to determine the phenomena that influence solute retention. Perhaps the two most important of these are the nature of the stationary phase and the composition of the mobile phase.

Reversed-phase models

Early reversed-phase retention studies listed solvents on an arbitrary scale, or "eluotropic series," showing their relative eluting strengths based on solvent polarity, P' [3-6], Hildebrand solubility parameter, δ [7], or solvent strength parameter, S [8-11]. Results from early experiments suggested that S may be invariant with solute type [7,11,12]. However, this was shown not to be the case by several researchers, including the author of the S scale, L.R. Snyder [12-15].

The solvophobic theory has been studied by several researchers [16-21]. This model suggests that hydrophobic solutes are energetically excluded from a polar solvent such as water due to the ordering of polar solvent molecules. The assumption is held by proponents of this model that the stationary phase has little impact on the retention of solutes [22]. Late experiments have shown, however, that attractive forces of a hydrophobic stationary phase for an organic solute can have a significant influence on retention [23]. Several studies have

reported results supporting or refuting the solvophobic theory [24-30]. Carr *et al.* [31] and Dorsey *et al.* [23,32] assert that the solvophobic theory does not adequately represent the retention process in reversed-phase chromatography.

Several retention models fall into the general category of linear solvation energy relationships (LSERs). Many of these studies show a linear correlation between chromatographic retention and one or more parameters of the dissolved analyte. Carr *et al.* have reviewed several retention models and describe LSERs as the correlation of a general solute property with three types of terms: a cavity term, a dipolar term, and hydrogen bond terms [33-41]. Other articles pertaining to LSERs report results correlating retention to solute parameters [42-67].

One of the goals of understanding retention is the ability to predict retention of a set of analytes and to manipulate separation conditions to optimize selectivity. A logical outcome of such study is to make use of computers to aid in method development. Several authors have reported the development of "expert systems" for this purpose [68-72].

Normal-phase models

Normal-phase chromatography can be traced back to Martin and Synge, who used silica gel as the stationary phase and an alcohol as the mobile phase [1,73]. Early liquid chromatographic techniques such as this were later called "normal-phase chromatography." Classical normal-phase separations were undertaken on bare silica gel or on bonded silicas. Many bonded silica-based stationary phases have been developed and are used for a large number of normal-phase as well as reversed-phase separations [16,74-93]. Alumina was also

a popular material in early separations [9,94,95] as well as more recent work [96-99].

Retention models in normal-phase chromatography are based on competitive adsorption between solute and solvent molecules for sorptive sites on the stationary phase [32]. Two of the most successful models for normal-phase chromatography are those advanced by Snyder *et al.* [32,100-103] and by Soczewinski *et al.* [32,104]. Both models describe a correlation between solute retention and solute-solvent competition for active sites on the stationary phase [32].

Stationary Phase Materials

Silica-based stationary phases

Although materials such as alumina [94-99] and zirconia [105-107] have been studied to some extent, the greatest number of HPLC separations have been carried out on some form of silica-based material. This is due in part to the high efficiency and structural stability of bonded silica columns. Fig. 1 shows a simplified diagram of a typical octadecylsilane bonded silica. These materials are made by reacting surface silanol groups of the silica bead with organosilanes to form siloxane bonds [22, pp.324]. When preparing a bonded silica such as octadecylsilane, some of the silanol groups are sterically hindered from reacting with the organosilane ligand. This leaves active silanols on the surface and contributes to surface heterogeneity. Also, other combinations of silicon and oxygen may exist on the surface, making it more difficult to produce a homogeneous stationary phase material. A heterogeneous surface can lead to a number of chromatographic problems including tailing, loss of resolution, and mixed-mode retention mechanisms [32]. Silica-based packings must

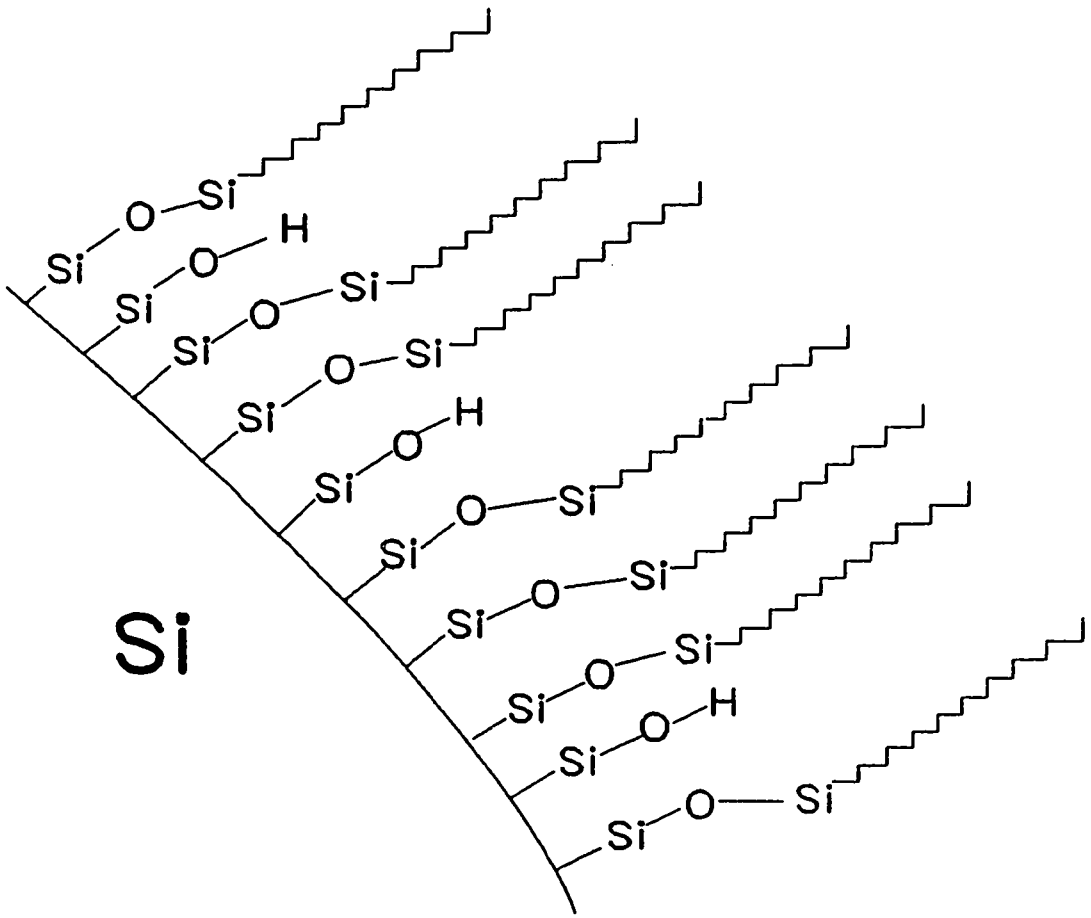

Fig. 1. Diagram of an octadecylsilane stationary phase.

be used in a pH range of about 2 to 8 in order to avoid degradation of the stationary phase.

Polystyrene-based stationary phases

Polystyrene-divinylbenzene (PS-DVB) packings have been studied as an alternative to silica-based materials. These packings offer the advantage of pH stability even at extreme values of pH. PS-DVB stationary phases also provide a relatively homogeneous surface. Its hydrophobicity makes it an excellent material for reversed-phase separations and, as discussed below, can also be used in the normal-phase mode, especially after derivatization of the surface with a polar group. Underivatized PS-DVB columns provide an additional advantage in reducing or eliminating peak tailing induced by residual silanol groups [108]. Although PS-DVB phases have the disadvantage of lower column efficiency, there are certain applications where it is beneficial to sacrifice lower efficiency for better pH stability and better peak shape. A diagram of polystyrene-divinylbenzene is given in Fig. 2.

Early work using polymer-based column packings for HPLC was undertaken by Peterson *et al.* [108-111]. These materials were not highly cross-linked and thus had gel-like characteristics. Rigid polymeric stationary phases were introduced in 1964 for open-column chromatography [112,113] and were first used in HPLC in 1985 [112, 114]. Moore used macroporous polystyrene highly cross-linked with divinylbenzene co-polymer [108,113].

Several studies have been devoted to the characterization and application of PS-DVB packings for HPLC [115-135]. Lloyd and co-workers used a microparticulate PS-DVB packing for reversed-phase HPLC [136]. Sulfonated PS-DVB was used by Moore [137] and by Scobell *et al.* [138] for HPLC, however, the beads were of low cross-linking and thus

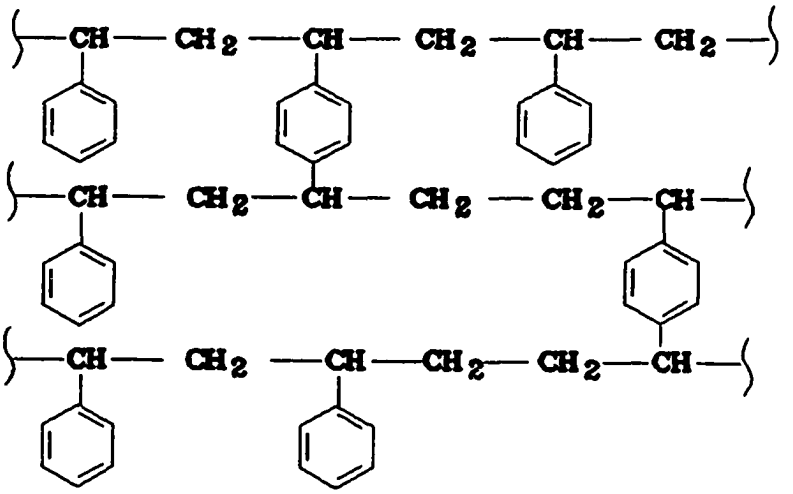

Fig. 2. Diagram of polystyrene-divinylbenzene.

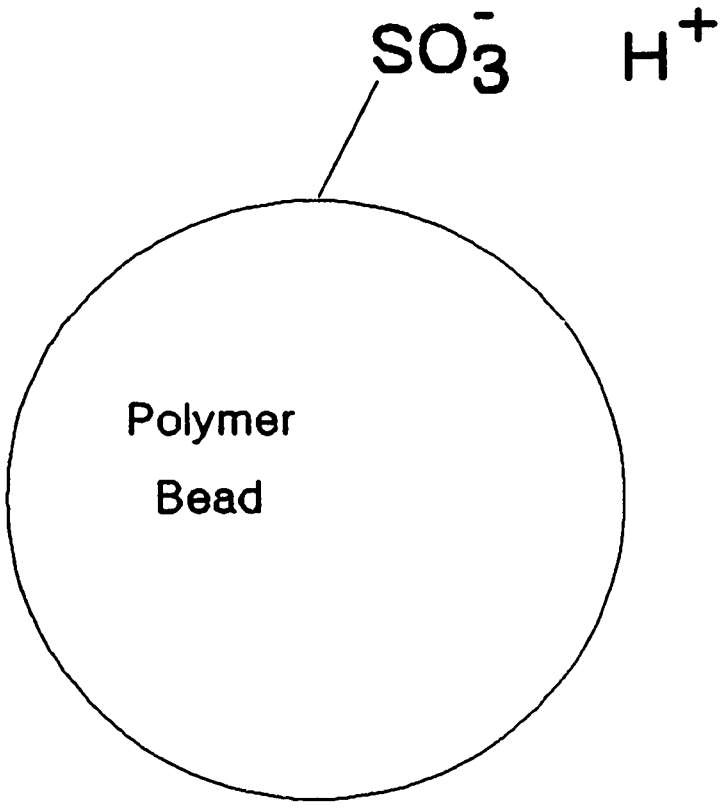

Fig. 3. Simplified diagram of sulfonated polystyrene-divinylbenzene.

required elevated temperatures for HPLC applications [108]. Fritz and co-workers used microparticulate (8 μm) lightly sulfonated PS-DVB beads to extract phenols and neutral compounds in solid-phase extraction experiments [139,140]. They found that the sulfonic acid group made the surface somewhat hydrophilic, which was useful for improving contact between dissolved solutes and the PS-DVB surface. Since the underivatized PS-DVB is initially hydrophobic, neutral organic compounds are readily sorbed onto the surface via hydrophobic interaction. Low-capacity sulfonation allows sorption of protonated bases following an ion-exchange mechanism [139]. Sulfonated polystyrene-divinylbenzene is depicted in the diagram in Fig. 3.

Having the advantages of pH stability and an initially homogeneous hydrophilic surface (no residual hydroxyl groups), it reasonable to expect that PS-DVB can be a useful material for HPLC separations. In this work, sulfonated and underivatized PS-DVB was used for the study of retention of polar and nonpolar organic compounds.

References

[1] J.S. Fritz and G.H. Schenk, *Quantitative Analytical Chemistry*, Fifth edition, Allyn and Bacon, Boston, 1987.

[2] L.R. Snyder and J.J. Kirkland, *Introduction to Modern Liquid Chromatography*, Second edition, John Wiley & Sons, New York, 1979.

[3] L. Rohrschneider, *Anal. Chem.*, 45 (1973) 1241.

[4] L.R. Snyder, *J. Chromatogr.*, 92 (1974) 223.

[5] L.R. Snyder, *J. Chromatogr. Sci.*, 16 (1978) 223.

[6] L.R. Snyder, *J. Chromatogr.*, 99 (1974) 745.

[7] J.H. Hildebrand and R.L. Scott, *The Solubility of Non-Electrolytes*, Dover Publications, New York, 1964.

[8] L.R. Snyder, J.W. Dolan and J.R. Gant, *J. Chromatogr.*, 165 (1979) 3.

[9] L.R. Snyder, *J. Chromatogr.*, 8 (1962) 178.

[10] L.R. Snyder, *J. Chromatogr.*, 11 (1963) 195.

[11] K. Karch, I. Sebastian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.

[12] T.L. Hafkenscheid and W. Tomlinson, *J. Chromatogr.*, 264 (1983) 47.

[13] P.J. Schoenmakers, H.A.H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.

[14] X. Geng and F.E. Regnier, *J. Chromatogr.*, 332 (1985) 147.

[15] M.A. Quarry, R.L. Grob, L.R. Snyder, J.W. Dolan and M.P. Rigney, *J. Chromatogr.*, 384 (1987) 163.

[16] C.F. Simpson (Ed.), *Techniques in Liquid Chromatography*, Wiley, New York, 1982.

[17] H. Engelhardt, J. Low, W. Eberhardt and M. Mauss, *Chromatographia*, 27 (1989) 535.

[18] Cs. Horváth, W.R. Melander and I Molnar, *J. Chromatogr.*, 125 (1976) 129.

[19] Cs. Horváth and W.R. Melander, *J. Chromatogr. Sci.*, 15 (1977) 393.

[20] W.R. Melander and Cs. Horváth, *Chromatographia*, 18 (1984) 353.

[21] H.J. Mockel, G. Welter and J. Melzer, *J. Chromatogr.*, 388 (1987) 255.

[22] C.F. Poole and S.K. Poole, *Chromatography Today*, Elsevier, Amsterdam, 1991.

[23] J.G. Dorsey, W.T. Cooper, J.F. Wheeler, H. Barth and J.P. Foley, *Anal. Chem.*, 66 (1994) 500R.

[24] P.W. Carr, L.C. Tan and J.H. Park, *J. Chromatogr. A*, 724 (1996) 1.

[25] K. Jinno, C. Kohrikawa, Y. Saito, J. Haiginaka, Y. Saito and M. Mifune. *J. Microcolumn Sep.*, 8 (1996) 13.

[26] V.A. Nzengung, E.A. Voudrias, P. Nkedikizza, J.M. Wampler and C.E. Weaver. *Environ. Sci. Tech.*, 30 (1996) 89.

[27] R.P. Bonar-Law, *J. Am. Chem. Soc.*, 117 (1995) 12397.

[28] G. Belfort, G.L. Altshuler and K.K. Thallam, *AIChE Journal*, 30 (1984) 197.

[29] N. Nirmalakhandan and R.E. Speece, *Environ. Sci. Tech.*, 24 (1990) 575.

[30] D.J. Cram, H.-J. Choi and J.A. Bryant, *J. Am. Chem. Soc.*, 114 (1992) 7748.

[31] P.W. Carr, J. Li, A.J. Dallas, D.l.Eikens and L.C. Tan, *J. Chromatogr. A*, 656 (1993) 113.

[32] J.G. Dorsey and W.T. Cooper, *Anal. Chem.*, 66 (1994) 857A.

[33] W.J. Cheong and P. W. Carr, *Anal. Chem.*, 61 (1989) 1524.

[34] L.C. Tan and P.W. Carr, *J. Chromatogr. A*, 656 (1993) 521.

[35] J.H. Park, P.W. Carr, M.H. Abraham, R.W. Taft, R.M. Doherty and M.J. Kamlet, *Chromatographia*, 25 (1988) 373.

[36] P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft and M.H. Abraham, *Anal. Chem.*, 57 (1985) 2971.

[37] R.W. Taft, J.-L.M. Abboud, M.J. Kamlet and M.H. Abraham, *J. Solution Chem.*, 14 (1985) 153.

[38] M.J. Kamlet, R.M. Doherty, J.-L.M. Abboud and R.W. Taft, *CHEMTECH*, 16 (1986) 566.

[39] M.J. Kamlet and R.W. Taft, *Acta Chem. Scand.*, B39 (1985) 611.

[40] M.J. Kamlet, J.-L.M. Abboud and R.W. Taft, *Prog. Phys. Org. Chem.* 13 (1981) 485.

[41] M.H. Abraham, R.W. Doherty, M.J. Kamlet and R.W. Taft, *Chem. Britain*, 75 (1986) 629.

[42] M.J. Kamlet, P.W.Carr, R.W. Taft and M.H. Abraham,*J. Am. Chem. Soc.*, 103 (1981) 6062.

[43] R.W. Taft, M.H. Abraham, R.M. Doherty and M.J. Kamlet, *J. Am. Chem. Soc.*, 107 (1985) 3105.

[44] M.H. Abraham, M.J. Kamlet and R.W. Taft, *Chem. Soc. Perkin Trans.*, 2 (1982) 923.

[45] M.J. Kamlet, J.-L.M. Abboud, M.H. Abraham and R.W. Taft, *J. Org. Chem.* 48 (1983) 2877.

[46] M.J. Kamlet, R.M. Doherty, R. W. Taft and M.H. Abraham, *J. Am. Chem. Soc.*, 105 (1983) 6741.

[47] M.J. Kamlet, M.H. Abraham, R.M. Doherty and R.W. Taft, *J. Am. Chem. Soc.*, 106 (1984) 464.

[48] J.W. Grate, R.A. McGill and D. Hilvert, *J. Am. Chem. Soc.*, 115 (1993) 8577.

[49] J.P. Hickey and D.R. Passino-Reader, *Environ. Sci. Tech.*, 25 (1991) 1753.

[50] Y. Marcus, *J. Phys. Chem.*, 95 (1991) 8886.

[51] M.J. Kamlet, R.M. Doherty and P. W. Carr, *Environ. Sci. Tech.*, 22 (1988) 503.

[52] Y. Marcus, M.J. Kamlet and R.W. Taft, *J. Phys. Chem.*, 92 (1988) 3613.

[53] M.J. Kammlet, R.M. Doherty and M.H. Abraham, *J. Phys. Chem.*, 92 (1988) 5244.

[54] M.J. Kamlet, R.M. Doherty and M.H. Abraham, *J. Phys. Chem.*, 91 (1987) 1996.

[55] J.N. Spencer, J.A. Andrefsky and J. Naghdi, *J. Phys. Chem.*, 91 (1987) 2959.

[56] Y. Marcus, *J. Phys. Chem.*, 91 (1987) 4422.

[57] J.-L. Abboud, G. Guiheneuf and M. Essfar, *J. Phys. Chem.*, 88 (1984) 4414.

[58] J. Barbosa, V. Sanznebot and I. Toro, *J. Chromatogr. A*, 725 (1996) 249.

[59] S.Y. Yang, J.G. Bumgarner, L.F.R. Kruk and M.G. Khaledi, *J. Chromatogr. A* 721 (1996) 323.

[60] J. Barbosa, J. Berges and V. Sanznebot, *J. Chromatogr. A*, 719 (1996) 27.

[61] J.A. Thomas, M.G. Hutchings, C.J. Jones and J.A. McCleverty, *Inorganic Chem.*, 35 (1996) 289.

[62] W.T. Tian and D.S. Ballantine, *J. Chromatogr. A*, 718 (1995) 357.

[63] J. Barbosa, R Berges and V. Sanznebot, *J. Liq. Chromatogr.*, 18 (1995) 3445.

[64] A.H. Lowrey and G.R. Famini, *Structural Chemistry*, 6 (1995) 357.

[65] A.H. Lowrey, C.J. Cramer, J.J. Urban and G.R. Famini, *Computers & Chemistry*, 19 (1995) 209.

[66] S.R. Sherman, D. Suleiman, M.J. Hait, M. Schiller, C.L. Liotta, C.A. Eckert, J. J. Li, P.W. Carr, R.B. Poe and S. C. Rutan, *J. Phys. Chem.*, 99 (1995) 11239.

[67] J.W. Grate, M.H. Abraham, C.M. Du, R.A. McGill and W.J. Shuely, *Langmuir*, 11 (1995) 2125.

[68] "Expert Systems for Liquid Chromatography," *Anal. Chem.*, 58 (1986) 1192A.

[69] R. Kaliszan, *Quantitative Structure Retention Relationships*, Pergamon Press, New York, 1986.

[70] K. Valkó, G Szabó, J. Rochrocht, K. Jemnitz and F. Darvas, *J. Chromatogr.* 485

(1990) 349.

[71] *HPLC-MetabolExpert* Documentation, Compu-drug, Ltd., Budapest, Hungary, 1989.

[72] P.P. Csokán, F. Darvas, F. Csizmadia and K. Valkó, *LC·GC*, 12 (1994) 40.

[73] A.J.P. Martin and R.L.M. Synge, *Biochem. J.* 35 (1941) 1358.

[74] K.K. Unger and B. Anspach, *Trends Anal. Chem.*, 6 (1987) 121.

[75] P.R. Brown and R.A. Hartwick (Eds.), *High Performance Liquid Chromatography*, Wiley, New York, 1989.

[76] K. Unger (Ed.), *Packings and Stationary Phases in Chromatographic Techniques*, Dekker, New York, 1989.

[77] K.M. Gooding and F.E. Regnier, *HPLC of Biological Macromolecules. Methods and Applications*, Dekker, New York, 1990.

[78] K.K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979.

[79] K.K. Unger, J.N. Kinkel, B. Anspach and H. Giesche, *J. Chromatogr.*, 296 (1984) 3.

[80] J. Nawrocki and B. Buszewski, *J. Chromatogr.*, 449 (1988) 1.

[81] J.G. Dorsey and K.A. Dill, *Chem. Rev.*, 89 (1989) 331.

[82] L.C. Sander and S.A. Wise, *CRC Crit. Revs. Anal. Chem.*, 18 (1987) 299.

[83] G. Szabo and K. Offenmuller, *Anal. Chem.*, 60 (1988) 213.

[84] R.S. Helburn, S.C. Rutan and J Pompano, *Anal. Chem.*, 66 (1994) 610.

[85] S. L. Abidi and T.L. Mounts, *J. Liq. Chromatogr.*, 19 (1996) 509.

[86] D.A. Barrett, V.A. Brown, P.N. Shaw, M.C. Davies, H. Ritchie and P. Ross, *J. Chrom. Sci.*, 34 (1996) 146.

[87] H. Engelhardt and P. Orth, *J. Liq. Chromatogr.*, 10 (1987) 1999.

[88] B. Buszewski, *Chromatographia*, 28 (1989) 574.

[89] T. Monde, T. Kmiusudi, T. Kuroda, K. Mikumo, T. Ohkawa and H. Fukube, *J. Chromatogr. A*, 722 (1996) 273.

[90] S.C. Churms, *J. Chromatogr. A*, 720 (1996) 75.

[91] H. Ohta, K. Jinno, Y. Saito, J.C. Fetzer, W.R. Biggs, J.J. Pesek, M.T. Matyska and Y.L. Chen, *Chromatographia*, 42 (1996) 56.

[92] Y. Saito, H. Ohta, H. Terasaki, Y. Katoh, H. Nagashima, K. Jinno and K. Itoh, *HRC J. High Res. Chromatogr.*, 18 (1995) 569.

[93] J.E. Ogara, B.A. Alden, T.H. Walter, J.S. Petersen, C.L. Niederlander and U.D. Neue, *Anal. Chem.*, 67 (1995) 3809.

[94] L.E. Snyder, *J. Chromatogr.*, 6 (1961) 22.

[95] C. Laurent, H.A.H. Villiet and L. de Galan, *Chromatographia*, 17 (1983) 253.

[96] E. Forgacs and T. Cserhati, *Analytical Lett.*, 29 (1996) 321.

[97] Z.H. Ji and I.L. Chang, *HRC J. High Res. Chromatogr.*, 19 (1996) 32.

[98] P.T. Jedrzejewski and L.T. Taylor, *J. Chromatogr. Sci.*, 33 (1995) 438.

[99] E. Forgacs and T. Cserhati, *Fresenius J. Anal. Chem.*, 351 (1995) 688.

[100] L.R. Snyder, *Principles of Adsorption Chromatography*, Dekker, New York, 1968.

[101] L.R. Snyder and T.C. Schunk, *Anal. Chem.*, 54 (1982) 1764.

[102] L.R. Snyder, J.L. Glajch and J.J. Kirkland, *J. Chromatogr.*, 218 (1981) 299.

[103] L.R. Snyder, *LC Magazine*, 1 (1983) 478.

[104] E. Soczewinski, *Anal. Chem.*, 41 (1969) 179.

[105] A.Kurganov, U. Trudinger, T. Isaeva and K. Unger, *Chromatographia*, 42 (1996) 217.

[106] M. Huckel, H.J. Wirth and M.T.W. Hearn, *J. Biochem. Biophys. Meth.*, 31 (1996) 165.

[107] H.J. Wirth and M.T.W. Hearn, *J. Chromatogr. A*, 711 (1995) 223.

[108] L.L. LLoyd, *J. Chromatogr.*, 544 (1991) 201.

[109] H.A. Sober and E.A. Peterson, *J. Am. Chem. Soc.*, 76 (1954) 1711.

[110] E.A. Peterson, *Cellulosic Ion Exchangers*, Elsevier, Amsterdam, 1970.

[111] J. Porath and P. Flodin, *Nature (London)*, 183 (1959) 1657.

[112] L.L. Lloyd and F.P. Warner, *J. Chromatogr.*, 512 (1990) 365.

[113] J.C.Moore, *J. Polym. Sci. Part A*, 2 (1964) 835.

[114] T.N. Tweeten, K.A. Tweeten, F.P. Warner and L.L. Lloyd, 5th International Symposium on HPLC of Proteins, Peptides, and Polynucleotides, Toronto, November 4-6, 1985.

[115] S. Coppi, A. Betti, C. Bighi, G.P. Cartoni and F. Coccioli, *J. Chromatogr.*, 442 (1988) 97.

[116] R.G. Baum, R. Saetre and F.F. Cantwell, *Anal. Chem.*, 52 (1980) 15.

[117] D.P. Lee and J.H. Kindsvater, *Anal. Chem.*, 52 (1980) 2425.

[118] D. Coppi, G. Blo and A. Betti, *J. Chromatogr.*, 388 (1987) 135.

[119] F. Nevejans and M. Verzele, *J. Chromatogr.*, 350 (1985) 145.

[120] F. Nevejans and M. Verzele, *J. Chromatogr.*, 406 (1987) 325.

[121] R.M. Smith and D.R. Garside, *J. Chromatogr.*, 407 (1987) 19.

[122] L.D. Bowers and S. Pedigo, *J. Chromatogr.*, 371 (1986) 243.

[123] H.W. Sturman, J. Kohler, S.O. Jansson and A. Litzen, *Chromatographia*, 23 (1987)

341.

[124] S. Coppe, A. Betti and S. Caldari, *J. Chromatogr.*, 395 (1987) 159.

[125] W.A. Moats, *J. Chromatogr.*, 366 (1986) 69.

[126] K.A. Tweeten and T.N. Tweeten, *J. Chromatogr.*, 359 (1986) 111.

[127] I.O. Kibwage, E. Roets, J. Hoogmartens and H. Vanderhaege, *J. Chromatogr.*, 330 (1985) 275.

[128] K Aramaki, T. Hanani and H.F. Walton, *Anal. Chem.*, 52 (1980) 1965.

[129] J. Bontemps, L. Bettendorf, J. Lombet, C. Granddfils, G. Dandrifosse, E. Schoffeniels, F. Nevejans and J. Crommen, *J. Chromatogr.*, 295 (1984) 486.

[130] D.P. Lee, *J. Chromatogr. Sci.*, 20 (1982) 203.

[131] Z. Iskandarani and D.J. Pietrzyk, *Anal. Chem.*, 53 (1981) 489.

[132] R.I. Greyson and A.M. Patch, *J. Chromatogr.*, 242 (1982) 349.

[133] W.A. Moats and L. Leskinen, *J. Chromatogr.*, 386 (1987) 79.

[134] L.C. Tan, P.W. Carr and J.M.J. Frechet, *Anal. Chem.*, 66 (1994) 450.

[135] R.S. Deinhammer, K. Shimazu and M.D. Porter, *Anal. Chem.*, 63 (1991) 1889.

[136] J.V. Dawkins, L.L. Lloyd and F.P. Warner, *J. Chromatogr.*, 352 (1986) 157.

[137] S. Moore, *J. Biol. Chem.*, 243 (1968) 6281.

[138] H.D. Scobell and K.M. Brobst, *J. Chromatogr.*, 212 (1981) 51.

[139] J.S. Fritz, P.J. Dumont and L.W. Schmidt, *J. Chromatogr. A*, 691 (1995) 133.

[140] P.J. Dumont and J.S. Fritz, *J. Chromatogr. A*, 691 (1995) 123.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH NONAQUEOUS SOLVENTS: RELATIVE ELUTION STRENGTH ON A POLYSTYRENE-DIVINYLBENZENE COLUMN

A paper accepted by *Journal of Chromatography A*

Thomas K. Chambers and James S. Fritz

Abstract

A polymeric resin-based stationary phase was used to study the retention of alkylphenols, alkylbenzenes, substituted benzenes, and fused-ring compounds in different eluents. Various 100% organic solvents were used as eluents in order to observe the relative ability of solvents to elute compounds of given chemical structure. Plots of log k' *versus* the number of carbons in a chain attached to the aromatic ring show linear correlations with slopes dependent on solvent type and solute functionality. Of the solvents studied, acetonitrile has the highest solvating ability for the benzene ring as well as for several functional groups. Ethanol has shown to have the strongest eluting power for the methylene group. The relative ability of several solvents to elute polynuclear aromatic hydrocarbons in solid phase extraction was determined. Functional group contribution (τ) was calculated for selected compounds and acceptable agreement was found in the correlation between experimental and predicted values for solute capacity factor.

Introduction

Retention of analytes in liquid chromatography is determined both by the properties of the stationary phase and by the mobile phase. The composition of the mobile phase plays

a critical role in determining the success of any HPLC separation. Most frequently, the mobile phase is a mixture of an organic solvent and water. Relatively complex theoretical treatments of mobile phase selectivity have tended to give way to simple models using easily measured parameters [1]. These include eluotropic series [2], solvatochromic comparison [3], and the solubility parameter scale [4], among others.

In the present work, the ability of solvents to solvate organic analytes was studied by measuring the capacity factors of substituted benzenes (C_6H_5X) in various media. These experiments were performed with a pure organic solvent as the mobile phase in order to eliminate any effects from different interactions of the organic solvent with water. Such interactions in mixed organic-water phases are a very real possibility. Methanol-water complexes in binary mixtures commonly used as the mobile phase in reversed-phase HPLC have already been shown to occur [5]. Scott *et al.* suggested that the capacity factor (k') is a function of the free (uncomplexed) methanol and not the total methanol concentration. Similar interactions between water and other organic solvents are definitely possible.

Although bonded-phase silica materials are generally used in reversed-phase HPLC, we selected polystyrene-divinylbenzene (PS/DVB) resins for several reasons. These provide larger surface area up to 800 m^2/g as opposed to round 250 m^2/g for bonded-phase silica. The larger surface area of PS/DVB gives larger capacity factors and permits measurement of solutes in pure solvents where the k' might be too small to measure accurately using silica particles. PS/DVB resins are also more robust in extreme pH conditions [1,6] and have a more homogeneous surface. Bonded-phase silicas have hydrocarbon chains extending from the surface and are apt to contain free silanol groups.

Another goal of this research was to provide better data regarding the elution of sample analytes in solid-phase extraction (SPE). Polymeric resins in mini-columns or membranes have been shown to be superior to cartridges containing silica-based sorbents for SPE [7,8]. However, only limited information has been available concerning the relative ability of pure organic solvents to elute analytes of different chemical structure from polymeric stationary phases.

Experimental

Reagents and chemicals

Solvents were obtained from Fisher Scientific (Pittsburgh, PA, USA) except absolute ethanol from Quantum Chemical (Newark, NJ, USA), and ethylene glycol monomethyl ether (2-methoxythanol, Sigma-Aldrich, Milwaukee, WI, USA). All solvents were of HPLC grade or reagent grade. Analyte compounds were purchased from Aldrich, Fisher, Mallinckrodt (St. Louis, MO, USA), or Eastman (Rochester, NY) and were of reagent grade except phenylacetaldehyde (90%).

Apparatus

The chromatograph consisted of a model 302 HPLC pump (Gilson, Middleton, WI, USA), model 783A UV absorbance detector (Applied Biosystems, Stone Mountain, GA, USA), model 7000 switcher (Rheodyne, Cotati, CA, USA, used as injector), Hitachi D-2000 Chromato-integrator (EM Science, Cherry Hill, NJ, USA), or model C-R3A Chromatopac integrator (Shimadzu, Kyoto, Japan), and a model LP-21 *Lo-Pulse* pulse dampener (Scientific

Systems Inc., State College, PA, USA). The column was 4.6 mm I.D. x 10 cm stainless steel slurry-packed in our laboratory with polystyrene-divinylbenzene beads of 5 μm average particle size and 80 Å to 100 Å average pore diameter (Sarasep, Santa Clara, CA, USA).

Procedure

Sample compounds were dissolved in methanol or acetonitrile. Chromatographic eluents were sparged with helium (Air Products, Des Moines, IA, USA) for 0.5 hr before transferring to the eluent reservoir. Pure organic solvents were used as mobile phases at a flow rate of 1 ml/min. Analyte concentrations ranged from 1 to 500 μg/mL and were injected using a 5 μL injection loop (Rheodyne, Cotati, CA, USA) individually or in sets, depending on resolution. Chromatographic peaks were detected using UV absorbance at 254 nm. Retention times were recorded with an integrator and the chromatographic capacity factor, k', was calculated for each analyte using the relation: $k' = (t_R - t_0)/t_0$, where t_R is the solute retention time and t_0 is the hold-up time. Column hold-up time (a function of the column void volume) was determined by measuring the time of the refractive index disturbance in the baseline following injection, as described by Dolan [9]. The values for hold-up time were checked by estimating the hold-up volume as roughly one-tenth of the column length and dividing this value by the flow rate to get the column hold-up time, as discussed in reference 9. Column temperature was held at 25° C with an Eldex Model III Temperature Control Unit (Eldex Laboratories, San Carlos, CA). Isocratic elution was used for all chromatography.

Results and Discussion

Retention as a function of carbon number

The linear relationship between the logarithm of the capacity factor and the carbon number within a homologous series is well known [10,11]. We studied the retention of several alkylbenzenes and several *p*-alkylphenols using each of three pure organic solvents as the mobile phase. The results in Figs. 1 and 2 show satisfactory linearity for plots of log k' as a function of the carbon number of the alkyl group. In both figures the log k' is lowest in acetonitrile (ACN) and highest in methanol (MeOH). This indicates that solvation of a benzene ring follows the order ACN > EtOH > MeOH.

The slope of log k' with carbon number for alkylbenzenes is the greatest in methanol and the smallest in ethanol (EtOH). This indicates that solvation of methylene and methyl groups follows the order EtOH > ACN > MeOH. For *p*-alkylphenols (Fig. 2) the slopes in methanol and acetonitrile are almost the same while the slope in ethanol is significantly lower. This shows again that ethanol provides the strongest solvation for methylene and methyl groups.

Comparison of log k' of an alkylphenol with that of the corresponding alkylbenzene gives a measure of the relative solvation of the phenolic -OH by each of the solvents studied. Phenolic solvation follows the order EtOH > MeOH > ACN. The stronger solvation by the two alcoholic solvents could be due to hydrogen bond formation between the alcohol and the phenol.

Elution and separation of polynuclear aromatic hydrocarbons

In solid-phase extraction it is customary to extract small amounts of organic solutes from predominantly aqueous samples onto a small column filled with a porous solid sorbent. The extracted substances are then eluted from the SPE column by a small volume of organic solvent. The literature contains many recipes for elution with single or mixed solvents. but numerical comparisons of the eluting ability of solvents are generally lacking.

We studied the ability of seven different solvents to elute polynuclear aromatic hydrocarbons (PAHs) from porous polystyrene resin columns by measuring their capacity factors. Several of the solvents eluted benzene so quickly that comparison of their relative eluting abilities was difficult. However, fused-ring aromatic compounds eluted more slowly and it was possible to compare the capacity factors obtained with the various solvents. The values obtained are given in Table 1.

These results show that tetrahydrofuran (THF) and methylene chloride (CH_2Cl_2) provide the lowest capacity factors. However, for practical SPE methylene chloride has the disadvantage of not being miscible with water and thereby possibly being less efficient for elution after having passed an aqueous sample through the column. Ethyl acetate also gives low capacity factors and has the property of being quite volatile when gas chromatography is used to separate and measure the eluted sample components.

The data in Table 1 show that methanol is a very poor solvent for eluting fused-ring aromatic hydrocarbons and that ethanol and acetonitrile are also rather weak eluents. The chromatogram in Fig. 3a shows that benzene and naphthalene are readily eluted by methanol but anthracene elutes much later in a very broad peak. Chrysene failed to elute in any

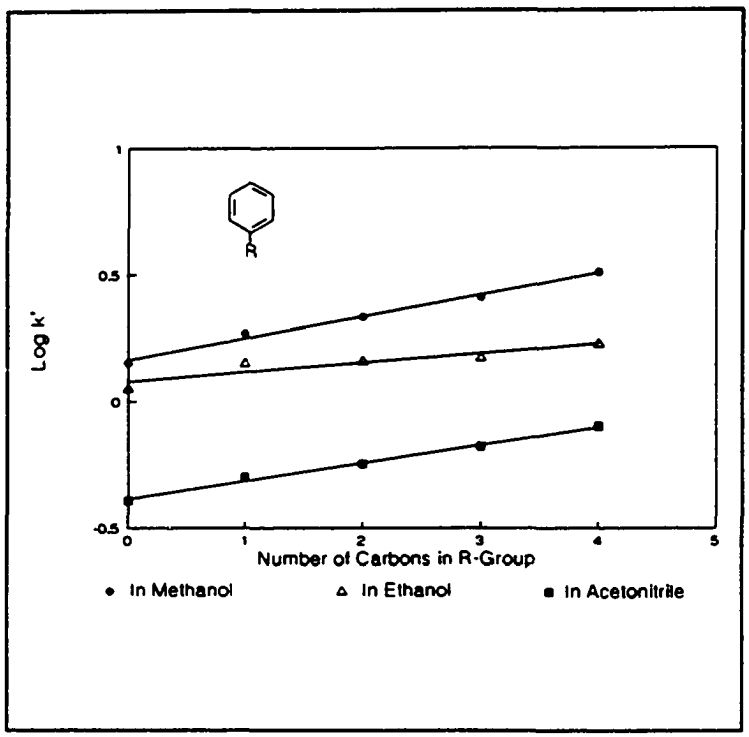

Fig. 1. Logarithmic capacity factor vs. carbon number for alkylbenzenes. Methanol: slope = 0.085, s.d = 0.005, r^2 = 0.991; Ethanol: slope = 0.038, s.d. = 0.009, r^2 = 0.858; Acetonitrile: slope = 0.071, s.d = 0.003, r^2 = 0.993.

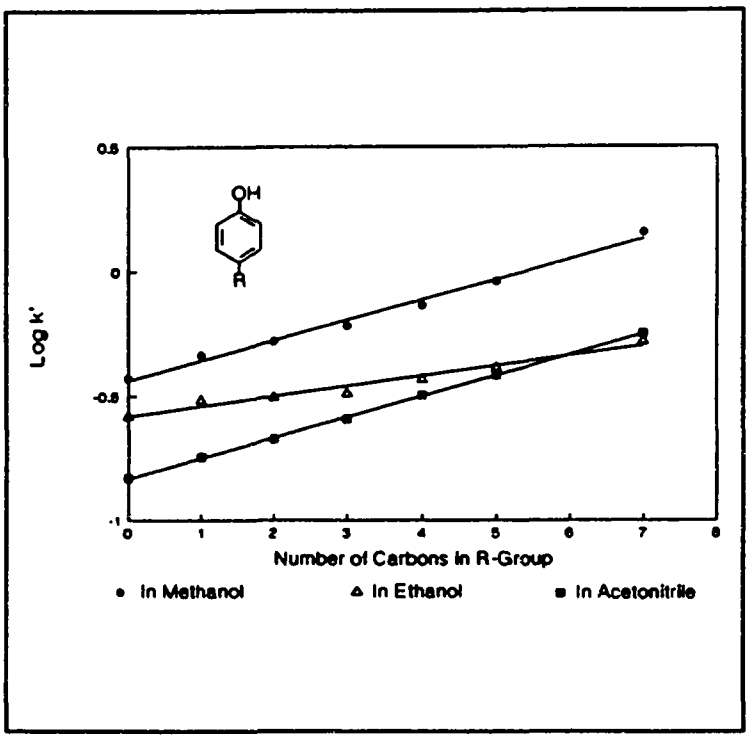

Fig. 2. Logarithmic capacity factor vs. carbon number for alkylphenols. Methanol: slope = 0.081, s.d. = 0.004, $r^2 = 0.990$; Ethanol: slope = 0.041, s.d. = 0.003, $r^2 = 0.968$; Acetonitrile: slope = 0.083, s.d. = 0.001, $r^2 = 0.999$.

Table 1
Chromatographic capacity factors for selected solutes in pure solvents

Solute	Solvent						
	MeOH	EtOH	ACN	THF	EGME	EtOAC	CH_2Cl_2
Benzene	1.44	1.13	0.40	-[a]	-[a]	-[a]	-[a]
Naphthalene	4.65	3.60	1.02	0.53	1.78	0.90	0.59
Anthracene	30.07	20.65	5.61	0.52	2.65	1.08	0.58
Chrysene	-[b]	-[b]	10.85	0.49	3.12	1.20	0.78

[a] Very low values.
[b] Very high values.

Solvents: Methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), tetrahydrofuran (THF), ethylene glycol monomethyl ether (EGME), ethyl acetate (EtOAc), methylene chloride (CH_2Cl_2)

reasonable time. These results suggest that PAH compounds with more than two ringscan be isolated selectively by solid-phase extraction. Smaller, more polar compounds in methanol will pass through a short, polymeric SPE column while the larger PAH compounds will be strongly retained. These, however, are quickly eluted by THF, ethyl acetate (EtOAc), or methylene chloride. We tried a simple experiment using about 50 mg of underivatized 5 μm d_p polystyrene-divinylbenzene in a 1 mL cartridge. A mixture of 75 μg/mL toluene, 1 μg/mL anthracene, and 5 μg/mL chrysene dissolved in ethanol and diluted with water was forced over the PS/DVB using positive air pressure. The packing was washed with 0.5 mL methanol to remove toluene. Tetrahydrofuran (1 mL) was used to elute the PAH compounds from the SPE packing. The effluent was collected in a volumetric flask, diluted to 10 mL with methanol, and analyzed by HPLC. Perylene was added as an internal standard and recoveries were calculated by comparison to a separate standard solution. The toluene peak was satisfactorily removed while anthracene and chrysene were recovered at 88% and 90%,

respectively.

A good chromatographic separation was obtained in acetonitrile for naphthalene, anthracene, and chrysene (Fig. 3b). Incomplete separation of the same mixture was obtained in ethylene glycol monomethyl ether (EGME, Fig. 3c). In ethyl acetate all three compounds were eluted together as a single early peak (Fig. 3d).

Functional group contributions to capacity factor

Several workers have studied the effect of a substituent on the retention of a given solute [12,13]. In many studies the functional group contribution is given by τ_X, defined by

$$\tau_X = \log k'_{R\text{-}X} - \log k'_{R\text{-}H} \qquad (1)$$

We measured the capacity factors of a number of substituted benzene and naphthalene compounds in methanol, ethanol, and acetonitrile. The capacity factors are given in Table 2 and τ values are listed in Table 3.

The capacity factors of several substituted compounds were predicted by adding the τ values for each substituent to the log k' of benzene or naphthalene. Table 4 compares the predicted and actual values of log k' for several compounds. For example, the difference in log k' values for chlorobenzene and benzene was calculated giving a τ of 0.15 for the chloro group in methanol. The log k' for chloronaphthalene was predicted by adding this same τ value to the log k' of naphthalene. Similar treatment was used for other compounds and reasonable agreement was obtained between predicted and actual values as shown in Fig. 4. The slope of this plot was very close to 1 and the intercept was 0.006. However, there are some obvious limitations to this approach. For example, the actual value for 2-nitrotoluene

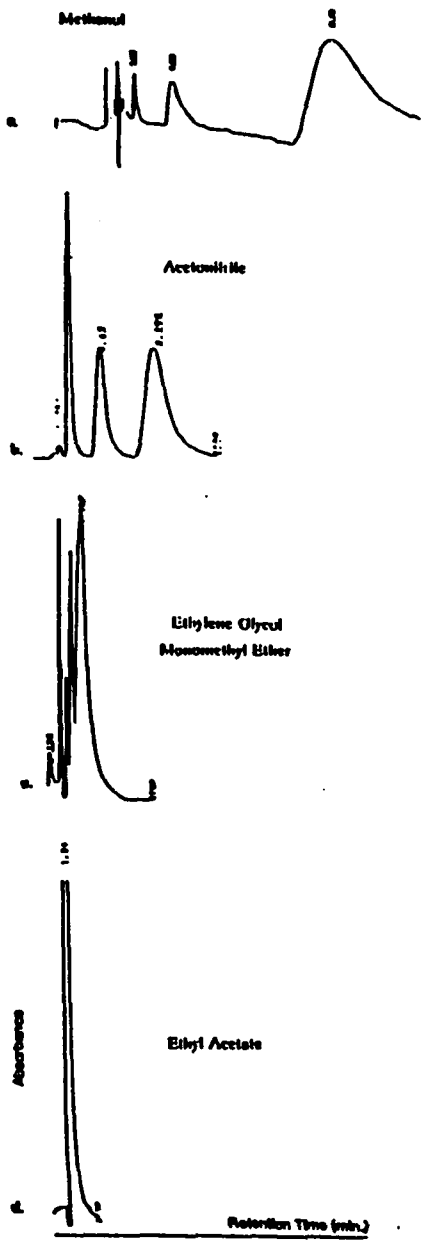

Fig. 3. Chromatograms of a. 150 μg/mL benzene, 25 μg/mL naphthalene, 20 μg/mL anthracene in 100% methanol eluent. b. 10 μg/mL naphthalene, 1 μg/mL anthracene, 10 μg/mL chrysene in 100% acetonitrile eluent. c. 10 μg/mL naphthalene, 1 μg/mL anthracene, 10 μg/mL chrysene in 100% ethylene glycol monomethyl ether eluent. d. 5 μg/mL naphthalene, 5 μg/mL anthracene, 5 μg/mL chrysene in 100% ethyl acetate eluent. Similar chart speeds.

is lower than the predicted value in each solvent. This could be due to weak interaction between the methyl hydrogen and the NO_2 group.

Most of the chromatographic peaks were sharp in the solvents studied. A number of separations are feasible, as illustrated by the separation of nitrobenzene and bromobenzene using pure methanol as the mobile phase (Fig. 5).

Table 5 provides a listing of the relative solvation of the various substituent groups by the solvents studied. The groups having the smallest (or most negative) τ values are considered to be the most strongly solvated. In general, polar groups are the most strongly solvated and more non-polar groups are less solvated, although the $-CH_2NH_2$ group seems to be an exception. The order of solvation varies among the solvents studied.

It is perhaps more meaningful to note for each analyte which solvent gives the greatest reduction in τ and therefore the greatest degree of solvation. Such a comparison can be made by observing values in Tables 1 and 2. Here we see that methanol gives the strongest solvation for the $-NH_2$; ethanol gives the strongest solvation for -OH, -Cl, -Br, and $-CH_2-$; acetonitrile most strongly solvates -COOH, -CHO, -CN, $-NO_2$, $-CH_2Cl$, $-CH_2Br$, $-CH_2CN$, $-CH_2SH$, benzene, and naphthalene. Further comparison of these substituted benzenes in the three solvents shows that the following groups are the most poorly solvated by each solvent: methanol: $-CH_2-$, $-CH_3$, -CN; ethanol: $-NH_2$, -COOH, CHO, $-NO_2$, $-CH_2CN$; acetonitrile: -OH, -Cl, -Br.

Table 2
Capacity factors for various compounds in organic solvents on polystyrene-divinylbenzene

Compound	MeOH	EtOH	ACN
Benzene	2.57	1.85	0.77
Chlorobenzene	1.99	1.24	0.60
Benzonitrile	1.16	-[a]	0.23
Nitrobenzene	1.80	1.63	0.30
Benzaldehyde	1.60	1.35	0.37
Benzoic acid	0.22	0.62	0.01
Aniline	0.60	0.66	0.22
Naphthalene	4.65	3.60	1.02
1-Chloronaphthalene	6.40	4.52	1.62
1-Naphthol	1.09	0.71	0.47
Quinoline	1.71	1.42	1.19
Phenethyl alcohol	0.46	0.29	0.16
3-Phenyl-1-propanol	0.54	0.31	0.21
Cinnamaldehyde	2.91	2.22	0.43
Phenylacetaldehyde	1.40	1.31	0.28
Cinnamyl alcohol	0.62	0.40	0.24
Benzylamine	4.20	3.25	1.01
Benzyl chloride	1.65	-[a]	0.39
Benzyl bromide	2.12	1.65	0.38
Benzyl cyanide	1.13	1.17	0.14
Toluenethiol	2.65	2.08	0.48
Benzyl acetate	1.69	1.50	0.23
Benzylacetone	1.59	1.35	0.23
Phenylacetic acid	0.34	-[a]	0.01
Benzene	1.42	1.13	0.40
Toluene	1.86	1.42	0.50

Ethylbenzene	2.15	1.45	0.57
Propylbenzene	2.57	1.49	0.66
Butylbenzene	3.23	1.69	0.80
Phenol	0.37	0.26	0.15
p-Cresol	0.46	0.31	0.18
4-Ethylphenol	0.53	0.32	0.21
4-Propylphenol	0.61	0.33	0.26
4-*n*-Butylphenol	0.73	0.37	0.32
4-*n*-Amylphenol	0.91	0.42	0.39
4-*n*-Heptylphenol	1.44	0.43	0.57
p-Chlorotoluene	2.67	1.82	0.75
2-Nitrotoluene	2.04	1.87	0.33

[a] Data not collected for these solute-solvent systems.

Table 3
Functional group contributions τ_x (Eq. 1) for the benzene ring

Functional group contribution, τ_x	Methanol	Ethanol	Acetonitrile
Br	0.26	0.21	0.28
Cl	0.15	0.04	0.17
CN	-0.09	-[a]	-0.25
NO_2	0.10	0.16	-0.14
CHO	0.05	0.08	-0.03
COOH	-0.81	-0.26	-1.69
NH_2	-0.37	-0.23	-0.26
CH_2NH_2	0.47	0.46	0.40
CH_2Cl	0.07	-[a]	-0.01
CH_2Br	0.17	0.17	-0.03
CH_2CN	-0.10	0.01	-0.46
CH_2SH	0.27	0.27	0.08
OH	-0.58	-0.63	-0.44
CH_2COCH_3	0.05	0.08	-0.25
CH_2COOCH_3	0.08	0.12	-0.24
CH_2COOH	-0.62	-[a]	-1.69
Carbons in alkyl chain Alkylbenzenes:			
C_1	0.12	0.10	0.09
C_2	0.18	0.11	0.15
C_3	0.26	0.12	0.22
C_4	0.36	0.18	0.30
Alkylphenols[b]:			
C_1	-0.49	-0.57	-0.35
C_2	-0.43	-0.55	-0.28

C_3	-0.37	-0.54	-0.20
C_4	-0.29	-0.48	-0.10
C_5	-0.19	-0.43	-0.02
C_7	0.01	-0.33	0.15

[a] Not measured.
[b] (E.g., log $k'_{p\text{-cresol}}$ - log k'_{benzene}).

Table 4
Predicted and actual log k' values using functional group contributions

Solvent	Compound	Functional group(s)	R-H	Log k'	
				Predicted	Actual
Methanol	*p*-Cresol	OH, CH_3	Benzene	-0.31	-0.33
	p-Cholrotoluene	Cl, CH_3	Benzene	0.42	0.43
	2-Nitrotoluene	NO_2, CH_3	Benzene	0.37	0.31
	1-Chloronaphthalene	Cl	Naphthalene	0.81	0.81
	1-Naphthol	OH	Naphthalene	0.09	0.04
Ethanol	*p*-Cresol	OH, CH_3	Benzene	-0.48	-0.51
	p-Chlorotoluene	Cl, CH_3	Benzene	0.19	0.26
	2-Nitrotoluene	NO_2, CH_3	Benzene	0.31	0.27
	1-Chloronaphthalene	Cl	Naphthalene	0.60	0.65
	1-Naphthol	OH	Naphthalene	-0.07	-0.15
Acetonitrile	*p*-Cresol	OH, CH_3	Benzene	-0.74	-0.74
	p-Chlorotoluene	Cl, CH_3	Benzene	-0.12	-0.13
	2-Nitrotoluene	NO_2, CH_3	Benzene	-0.44	-0.48
	1-Chloronaphthalene	Cl	Naphthalene	0.18	0.21
	1-Naphthol	OH	Naphthalene	-0.43	-0.32

Based on functional group contribution (τ) from Table 1.3. Log $k'_{\text{R-X(predicted)}} = \log k'_{\text{(R-H)}} + \tau_{X1} + \tau_{X2} + \cdots$.

Table 5
Order of solvation from top to bottom: (best solvated to least solvated) of selected functional groups in three 100% organic eluents

Methanol	Ethanol	Acetonitrile
COOH	OH	COOH
CH_2COOH	COOH	CH_2COOH
OH	NH_2	CH_2CN
NH_2	Benzene	OH
CH_2CN	CH_2CN	NH_2
CN	Cl	CH_2COCH_3
Benzene	CH_2COCH_3	CH_2OOCH_3
CH_2COCH_3	CHO	CN
CHO	CH_3	NO_2
CH_2Cl	CH_2OOCH_3	CHO
CH_2OOCCH_3	NO_2	CH_2Br
NO_2	CH_2Br	CH_2Cl
CH_3	Br	Benzene
Cl	CH_2SH	CH_2SH
CH_2Br	CH_2NH_2	CH_3
Br	Naphthalene	Cl
CH_2SH		Br
CH_2NH_2		CH_2NH_2
Naphthalene		Naphthalene

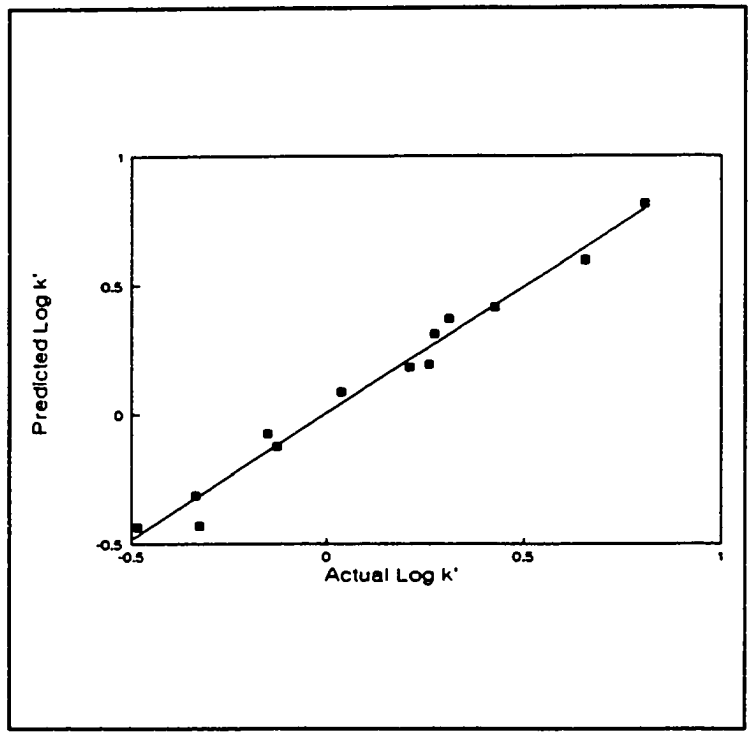

Fig. 4. Correlation of predicted and actual log k′ based on functional group contribution to capacity factor. Slope = 0.98, s.d. = 0.03, intercept = 0.006, s.d 0.05, r^2 = 0.987.

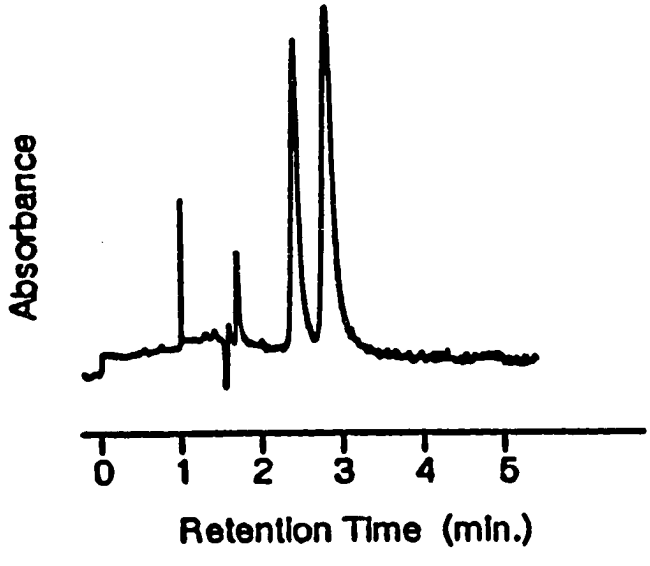

Fig. 5. Separation of 1 μg/mL nitrobenzene and 100 μg/mL bromobenzene in 100% methanol on 5μm PS-DVB.

Conclusions

By comparing the capacity factors of substituted benzenes and naphthalenes with that of the parent hydrocarbon, it has been possible to measure the relative degrees of solvation of the substituent groups by various organic solvents. The use of pure solvents as the mobile phase in HPLC avoids uncertainties related to solvent-water interactions that may occur when organic-water mixtures are used [5]. Measurement of the capacity factors of PAH compounds in each of seven pure solvents has given a numerical comparison of the relative eluting efficiencies of these solvents in solid-phase extraction. Methanol and ethanol have been shown to be very poor for eluting PAHs from SPE columns. However, advantage can be taken of this fact to selectively isolate PAHs from other organics by SPE in methanol or ethanol with subsequent rapid elution by a more effective solvent such as ethyl acetate or methylene chloride.

Acknowledgements

We thank Doug Gjerde of Sarasep, Inc. for resins used in this research. This work was supported by the Office of Basic Energy Sciences, U.S. Department of Energy and in part by a grant from the 3M Co., St. Paul, MN. The work was performed at the Ames Laboratory at Iowa State University. Ames Laboratory is operated for the U.S. Department of Energy under Contract No. W-7405-Eng-82.

References

[1] C.F. Poole and S.K. Poole, *Chromatography Today*, Elsevier, Amsterdam, 1991, p. 333-339.

[2] L.R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.

[3] J.E. Brady, D. Bjorkman, C.D. Herter, and P.W. Carr, *Anal. Chem.*, 56 (1984) 278.

[4] J.H. Hildebrand and R.L. Scott, *The Solubility of Nonelectrolytes*, 3rd ed., Dover, New York, 1964.

[5] E.D. Katz, C.H. Lochmüller, and R.P.W. Scott, *Anal. Chem.*, 61 (1989) 349.

[6] I. Liška, J. Krupčík, and P.A. Leclercq, *J. High Res. Chromatogr.*, 12 (1989) 577.

[7] M.C. Hennion and V. Coquart, *J. Chromatogr.*, 642 (1993) 211.

[8] S. Bitteur and R. Rosset, *J. Chromatogr.*, 394 (1987) 279.

[9] J.W. Dolan, *LC•GC*, 13(1) (1995) 24.

[10] G.E. Berendsen, P.J. Shoenmakers, L. de Galan, G. Vigh, Z. Varga-Puchony, and J. Inczedy, *J. Liq. Chromatogr.*, 3(11) (1980) 1669.

[11] R.F. Strasburg, D.M. Scott and J.S. Fritz, *Anal. Chim. Acta*, 215 (1988) 311.

[12] C.M. Riley, E. Tomlinson, and T.M. Jeffries, *J. Chromatogr.*, 185 (1979) 197.

[13] R.M. Smith, *J. Chromatogr.*, 656 (1993) 381.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH NON-AQUEOUS BINARY SOLVENT MIXTURES

A paper submitted to *Journal of Chromatography*

Thomas K. Chambers and James S. Fritz

Abstract

Three nonaqueous solvent systems have been studied for high performance liquid chromatography on a polystyrene-divinylbenzene column. Binary mixtures of methanol/ethyl acetate, acetonitrile/ethyl acetate, and hexane/1-chlorobutane were used as mobile phases with compositions varying from 0 to 100% volume fraction of the strong solvent. Plots of the log of the capacity factor *versus* the volume fraction of the strong solvent showed a quadratic dependence. For the hexane/chlorobutane system, a decrease in retention was observed for an *increase* in the polarity of the mobile phase, contrary to what is commonly observed in aqueous/organic solvent systems. *p*-Cresol was found to elute *after* PAH compounds in the hexane/1-chlorobutane system, the opposite of what is expected for a reversed-phase system using a hydrophobic stationary phase.

Introduction

Reversed-phase liquid chromatography is commonly carried out with bonded-phase silica particles as the stationary phase and an organic/aqueous mixture as the mobile phase. It was shown in a recent paper that practical HPLC separations can also be performed with an organic solvent containing little, if any, water as the mobile phase, provided porous polystyrene-divinylbenzene packings are used as the stationary phase instead of bonded-phase

silica [1].

The use of nonaqueous mobile phases in many cases allows the direct measurement of solute capacity factor at 0% strong organic modifier (k'_0). In this way, the value for k'_0 can be measured experimentally rather than extrapolated using models based on single- or multi-parameter scales [2-6] developed to correlate retention as a function of solvent composition.

In the present work, HPLC experiments were performed using binary mixtures of organic solvents. No water was present other than the small amounts that might accompany the organic solvents. The solvents were chosen so that one solvent (B) was a more effective solvating agent than the other (A) for the sample solutes. Thus, the capacity factors (k') decreased as a higher proportion of B was added to the mobile phase.

The organic solvents were also selected so that capacity factors of the solutes were low enough to be measured in each of the pure solvents as well as over the entire range of A/B mixtures. In the more conventional water/organic mobile phases used in HPLC, the capacity factors are often too high to be measured experimentally in pure water or in mixtures containing only a small proportion of the organic solvent.

One goal of this research was to demonstrate that a binary mixtures of two organic solvents can be used as the mobile phase in HPLC. Another goal was to study the dependence of the capacity factors of various analytes on mobile phase composition over the entire range of A/B solvent concentrations.

Experimental

Reagents and chemicals

Acetonitrile, methanol, ethyl acetate, and hexane were obtained from Fisher Scientific (Pittsburgh, PA, USA) and were HPLC grade or reagent grade. 1-Chlorobutane was obtained from Sigma-Aldrich (Milwaukee, WI, USA), and was HPLC grade. Analyte compounds were purchased from Aldrich, Fisher, Mallinckrodt (St. Louis, MO, USA), or from Eastman Kodak (Rochester, NY, USA) and were of analytical reagent grade.

Apparatus

The chromatograph consisted of a Gilson model 302 pump (Gilson, Middleton, WI, USA), a model 783A UV absorbance detector (Applied Biosystems, Stone mountain, GA, USA), model 7000 switcher (Rheodyne, Cotati, CA, USA, used as injector), Hitachi model D-2000 *Chromato-Integrator* (EM Science, Cherry Hill, NJ, USA), or Shimadzu model C-R3A integrator (Shimadzu, Kyoto, Japan), and a model LP-21 *Lo-Pulse* pulse dampener (Scientific Systems Inc., State College, PA, USA). The column was 4.6 mm I.D. x 10 cm stainless steel slurry-packed in our laboratory with 55% cross-linked polystyrene-divinylbenzene (PS-DVB) beads of 5 μm average particle size and 80 Å to 100 Å average pore diameter (Sarasep, Santa Clara, CA, USA).

Procedure

Sample compounds were dissolved in methanol or acetonitrile. Chromatographic eluents were sparged with helium (Air Products, Des Moines, IA, USA) for 0.5 hr before

transferring to the eluent reservoir. Binary organic solvent mixtures were used as mobile phases at a flow rate of 1 mL/min. Analyte concentrations ranged from 1 to 500 μg/mL and were injected using a 5μL injection loop (Rheodyne, Cotati, CA, USA). Chromatographic peaks were detected using UV absorbance at 254 nm. Retention times were recorded with an integrator and the chromatographic capacity factor, k', was calculated for each analyte using the relation: $k' = (t_R - t_o)/t_0$ where t_R is the solute retention time and t_0 is the hold-up time. Column hold-up time was determined by measuring the time of the refractive index disturbance in the baseline following injection, as described by Dolan [7], or by measuring the retention time of bromide ion. The values for hold-up time were checked by estimating the hold-up volume as roughly one-tenth of the column length and dividing this value by the flow rate to get the column hold-up time, as discussed in reference 7. All chromatograms were generated isocratically and retention data were reported as the average of at least three injections.

Results and Discussion

Acetonitrile/ethyl acetate system

It has been shown that ethyl acetate is an appreciably better solvent than acetonitrile for eluting polynuclear aromatic hydrocarbon (PAH) compounds from an HPLC column packed with porous PS-DVB particles [1]. Additional experiments revealed that chromatographic separations are feasible with mixed acetonitrile/ethyl acetate mobile phases and that capacity factors of organic solutes can be accurately measured.

Figs. 1 and 2 show plots of log k' *versus* mobile phase composition for chrysene and

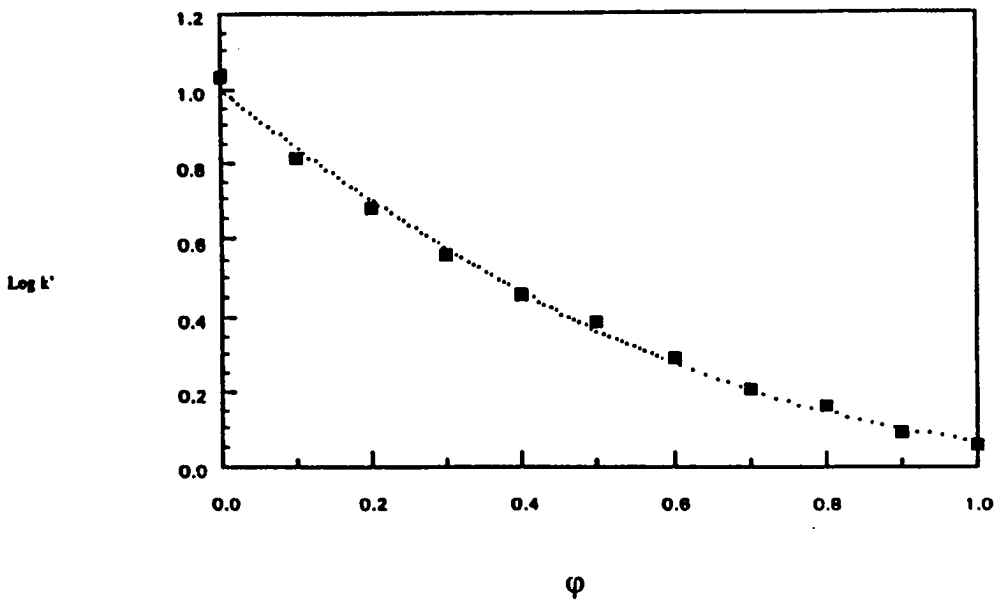

Fig. 1. Logarithm of the capacity factor of chrysene *versus* volume fraction ethyl acetate (φ) in acetonitrile/ethyl acetate mobile phase. UV detection at 254 nm. Flow rate = 1 mL min.

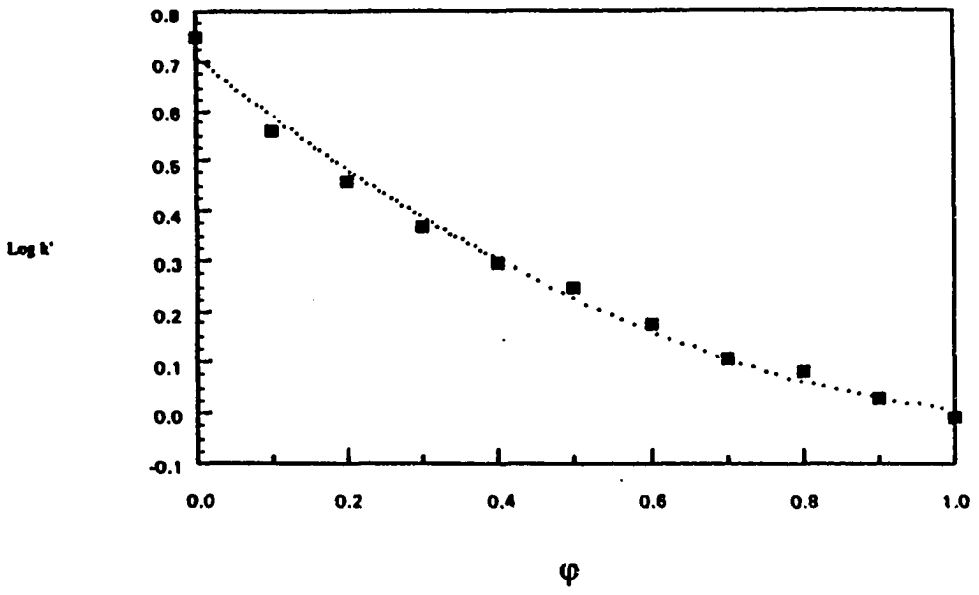

Fig. 2. Logarithm of the capacity factor of anthracene *versus* volume fraction ethyl acetate in acetonitrile/ethyl acetate mobile phase. Similar conditions as Fig. 1.

anthracene, respectively. In these figures, φ represents the volume fraction of ethyl acetate in the acetonitrile/ethyl acetate system. It will be seen that these plots are not linear in any region of mobile phase composition. This is in contrast to similar plots for aqueous/organic solvent mixtures which are often almost linear in some composition range. Using commercial software (PSIPLOT, Poly Software International, Salt Lake City, UT, USA) and a personal computer, the data in Figs. 1 and 2 were found to fit a mathematical equation of the type

$$\log k' = A\varphi^2 + B\varphi + C \qquad (1)$$

where A, B, and C are parameters for the fit. The filled points in Figs. 1 and 2 are experimental data points while the dotted lines are from Eq. (1) after computer calculation of the constants A, B, and C. Other groups have shown quadratic dependence for chromatographic data [8-11]. Schoenmakers *et al.* [9] reported values for A, B, and C parameters for 32 compounds in methanol/water, acetonitrile/water, and tetrahydrofuran/water on a bonded-phase column. However, in traditional reversed-phase systems, the value for k'_0 (k' at $\varphi = 0$) can only be extrapolated, which becomes hazardous at extreme values of φ [5].

Other solutes followed the same pattern as chrysene and anthracene. Table 1 lists values of the empirical constants and retention data for the solutes tested. Reasonable correlation was obtained between the experimental and calculated plots, as expressed by the correlation coefficients listed in the table.

Table 1
Empirical constants for quadratic fit (at 95% confidence level) and retention data as a function of volume fraction ethyl acetate in nonaqueous acetonitrile/ethyl acetate mobile phase mixture

Solute		Fit Parameter	S.D.	φ	k'	Log k'
Naphthalene	*A*	0.37	0.09	0.0	2.52	0.40
R^2 = 0.988	*B*	-0.79	0.09	0.1	1.77	0.25
	C	0.36	0.02	0.2	1.55	0.19
				0.3	1.36	0.14
				0.4	1.25	0.10
				0.5	1.20	0.08
				0.6	1.06	0.03
				0.7	0.95	-0.02
				0.8	0.94	-0.02
				0.9	0.86	-0.06
				1.0	0.83	-0.08
Anthracene	*A*	0.53	0.08	0.0	5.61	0.75
r^2 = 0.996	*B*	-1.23	0.09	0.1	3.62	0.56
	C	0.71	0.02	0.2	2.88	0.46
				0.3	2.34	0.37
				0.4	1.98	0.30
				0.5	1.76	0.25
				0.6	1.49	0.17
				0.7	1.28	0.11
				0.8	1.21	0.08
				0.9	1.07	0.03
				1.0	0.98	-0.01

Chrysene	*A*	0.69	0.07	0.0	10.85	1.04
r^2 = 0.998	*B*	-1.62	0.08	0.1	6.55	0.82
	C	1.00	0.02	0.2	4.78	0.68
				0.3	3.64	0.56
				0.4	2.87	0.46
				0.5	2.41	0.38
				0.6	1.94	0.29
				0.7	1.61	0.21
				0.8	1.44	0.16
				0.9	1.24	0.09
				1.0	1.15	0.05

Methanol/ethyl acetate system

Capacity factors of the sample solutes tested were higher in this system than in the acetonitrile/ethyl acetate system. With chrysene, the capacity factor was too high to measure in pure methanol, but addition of ethyl acetate lowered k' to measurable values over most of the mobile phase range. A plot of log k' *versus* φ is shown in Fig. 3.

In this case, fitting the experimental points to a quadratic equation is complicated by the fact that the value for k' at $\varphi = 0$ (the C term in Eq. (1)) must be estimated. Such an extrapolation introduces an additional source of error [5]. Nevertheless, a reasonable fit of the experimental data into a quadratic equation was obtained. Data for capacity factors as a function of φ and the quadratic constants A, B, and C are given in Table 2.

Hexane/1-chlorobutane system

In reversed-phase chromatography, the retention of an organic solute is usually found to decrease as the polarity of the mobile phase decreases. Several studies [11-14] have described RPLC retention as the result of two primary effects, one the energy required to make a cavity among solvent molecules, and the other being the ability of the solute to hydrogen-bond with the polar component of the mobile phase, where the solute acts as a hydrogen-bond acceptor and the solvent acts as a hydrogen-bond donor. In the present study, the results for the hexane/1-chlorobutane system show the opposite effect: retention decreases with an *increase* in the polarity of the mobile phase. In this case, any hydrogen bonds forming between a solute and chlorobenzene would be extremely weak. Thus, the decrease in retention due to increased hydrogen bonding of the solute with the B component is

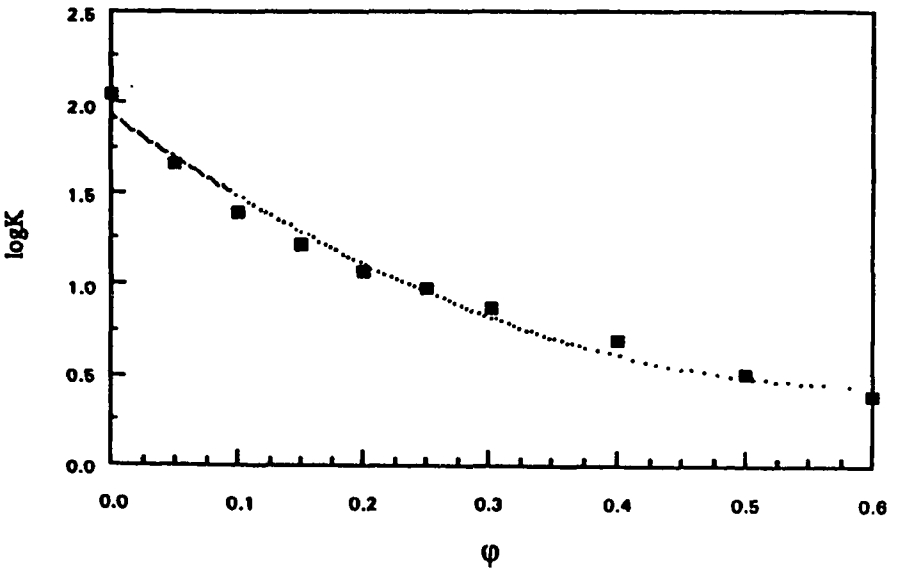

Fig. 3. Logarithm of the capacity factor of chrysene *versus* volume fraction ethyl acetate in methanol/ethyl acetate mobile phase. UV detection at 254 nm. Flow rate = 1 mL/min.

Table 2
Empirical constants for quadratic fit (at 95% confidence level) and retention data as a function of volume fraction ethyl acetate in nonaqueous methanol/ethyl acetate mobile phase mixture

Solute		Fit Parameter	S.D.	φ	k'	Log k'
Naphthalene	*A*	1.15	0.26	0.0	7.63	0.88
R^2 = 0.976	*B*	-2.01	0.28	0.1	-	-
	C	0.83	0.07	0.2	-	-
				0.3	1.53	0.19
				0.4	1.62	0.21
				0.5	1.38	0.14
				0.6	1.21	0.08
				0.7	1.12	0.05
				0.8	0.92	-0.03
				0.9	0.87	-0.06
				1.0	0.86	-0.07
Anthracene	*A*	1.51	0.22	0.0	29.79	1.47
R^2 = 0.992	*B*	-2.83	0.22	0.1	10.68	1.03
	C	1.36	0.05	0.2	5.83	0.77
				0.3	4.33	0.64
				0.4	2.96	0.47
				0.5	2.23	0.35
				0.6	1.78	0.25
				0.7	1.52	0.18
				0.8	1.19	0.07
				0.9	1.09	0.04
				1.0	1.04	0.02

Chrysene	*A*	4.05	0.77	0.0	112.20[a]	2.05
R^2 = 0.991	*B*	-4.91	0.47	0.05	45.71[a]	1.66
	C	1.92	0.06	0.10	24.35	1.39
				0.15	16.21[b]	1.21
				0.20	11.50	1.07
				0.25	9.54[b]	0.98
				0.30	7.76	0.88
				0.40	4.86	0.69
				0.50	3.24	0.51
				0.60	2.40	0.39

[a]Estimated by extrapolation of a curve fit to the data.
[b]Interpolated from a curve fit to the data.

minimal.

This system is similar to the mobile phases commonly used in normal-phase chromatography. In this type of HPLC, hexane is often referred to as the "carrier solvent" and a second, polar solvent in the mixture is called the "modifier." Usually, the modifier is considerably more polar than chlorobutane. In conventional normal-phase chromatography, a very hydrophilic stationary phase packing material is generally used. The use of a relatively non-polar polymer bead with a solvent mixture of low polarity is quite uncommon, but can be used to acquire traditionally elusive values such as k'_0, which is often very difficult to measure in a reasonable time in aqueous/organic mixtures.

For several sample solutes tested, plots of log k' as a function of φ (the fraction of chlorobenzene in the mobile phase) again give a quadratic curve (Fig. 4). Numerical data for log k' *versus* φ are given in Table 3, along with values for the parameters *A*, *B*, and *C*.

The elution order of organic solutes in this system is interesting. For any given mobile phase composition, the capacity factors of aromatic hydrocarbons increase with increasing bulk of the solute molecule. In conventional, reversed-phase chromatography, small compounds elute before larger, polycyclic molecules. In the present system, however, a small, polar solute such as *p*-cresol elutes later than any of the PAH compounds, as seen in Table 3. This is indicative of normal-phase chromatography in which the polar compounds are last to elute. A separation of toluene, phenethyl alcohol, and *p*-cresol in 30% hexane : 70% 1-chlorobutane is shown in Fig. 5.

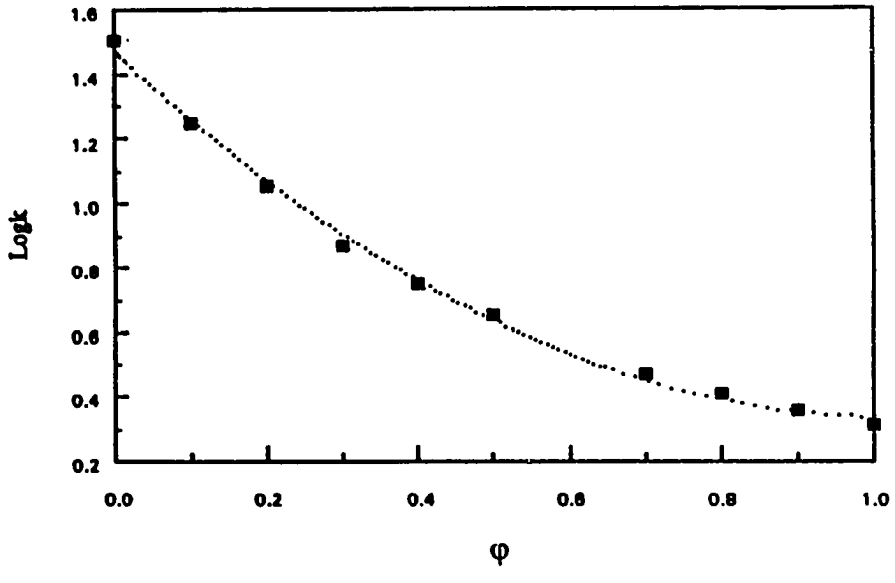

Fig. 4. Logarithm of the capacity factor of *p*-cresol *versus* volume fraction 1-chlorobutane in hexane/1-chlorobutane mobile phase. UV detection at 254 nm. Flow rate = 1 mL/min.

Table 3
Empirical constants for quadratic fit (at 95% confidence level) and retention data as a function of volume fraction 1-chlorobutane in nonaqueous hexane/1-chlorobutane mobile phase mixture

Solute		Fit Parameter	S.D.	φ	k'	Log k'
Toluene	*A*	0.27	0.04	0.0	1.33	0.12
R^2 = 0.992	*B*	-0.49	0.04	0.1	1.11	0.05
	C	0.10	0.01	0.2	1.01	0.01
				0.3	0.94	-0.03
				0.4	0.89	-0.05
				0.5	0.84	-0.07
				0.6	0.83	-0.08
				0.7	0.79	-0.10
				0.8	0.79	-0.10
				0.9	0.76	-0.12
				1.0	0.76	-0.12
Naphthalene	*A*	0.48	0.04	0.0	2.60	0.41
R^2 = 0.998	*B*	-0.93	0.04	0.1	2.00	0.30
	C	0.40	0.01	0.2	1.66	0.22
				0.3	1.43	0.15
				0.4	1.27	0.10
				0.5	1.16	0.06
				0.7	0.99	-0.01
				0.9	0.90	-0.05
				1.0	0.86	-0.06

Solute		Fit Parameter	S.D.	φ	k'	Log k'
Anthracene	*A*	0.91	0.08	0.0	6.34	0.80
R^2 = 0.998	*B*	-1.61	0.08	0.1	4.09	0.61
	C	0.78	0.02	0.2	2.99	0.48
				0.3	2.30	0.36
				0.4	1.90	0.28
				0.5	1.63	0.21
				0.7	1.26	0.10
				0.9	1.23	0.09
				1.0	1.13	0.05
Chrysene	*A*	1.08	0.10	0.0	13.71	1.14
R^2 = 0.998	*B*	-2.11	0.10	0.1	7.74	0.89
	C	1.11	0.02	0.2	5.06	0.70
				0.3	3.56	0.55
				0.4	2.71	0.43
				0.5	2.19	0.34
				0.7	1.51	0.18
				0.9	1.23	0.09
				1.0	1.13	0.05

Solute		Fit Parameter	S.D.	φ	k'	Log k'
Phenethyl Alcohol	*A*	0.92	0.10	0.0	11.29	1.05
$R^2 = 0.995$	*B*	-1.80	0.10	0.1	6.23	0.79
	C	1.00	0.03	0.2	4.36	0.64
				0.3	3.29	0.52
				0.4	2.63	0.42
				0.5	2.19	0.34
				0.7	1.66	0.22
				0.8	1.50	0.18
				0.9	1.34	0.13
				1.0	1.23	0.09
p-Cresol						
$R^2 = 0.998$	*A*	1.08	0.09	0.0	31.71	1.50
	B	-2.21	0.09	0.1	17.97	1.25
	C	1.47	0.02	0.2	11.10	1.05
				0.3	7.46	0.87
				0.4	5.67	0.75
				0.5	4.43	0.65
				0.7	2.93	0.47
				0.8	2.57	0.41
				0.9	2.30	0.36
				1.0	2.04	0.31

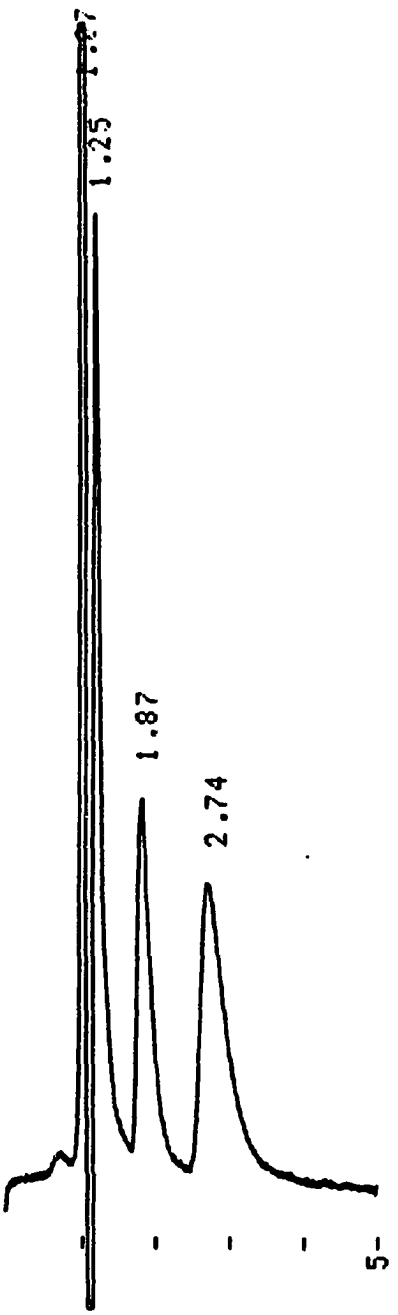

Fig. 5. Chromatogram of toluene, phenethyl alcohol, and *p*-cresol. Mobile phase: 30% hexane : 70% 1-chlorobutane. Polystyrene-divinylbenzene column, 5 μm d_p, 55% cross-linked, 80-100 Å average pore size, 10 cm x 4.6 mm. UV absorbance detection at 254 nm.

Retention as a function of mobile phase composition

It is interesting to note that experimental values for B in Eq. (1) are negative. B values reported by Schoenmakers and co-workers [9] for methanol/water, acetonitrile/water, and tetrahydrofuran/water systems were also negative for the 32 solutes studied. From Eq. (1) we see that as φ gets very small, the A term becomes less operable and the B term becomes dominant. In Figs. 1-4, the curves approach linearity for a very short segment corresponding to low values of φ. This line represents the effect of the B term on retention. At higher values of φ, the curvature is greater and less able to be approximated by a straight line. Similarly, effect of the A term can be represented by a quadratic curve with a positive coefficient. When $\varphi = 0$, $\log k'$ is equal to C, the intercept, from which k'_0 can be derived. A hypothetical graph of the A, B, and C terms of Eq. (1) is depicted in Fig. 6. When typical parameters are inserted for A, B, and C, the sum of the effects simulates an experimental curve for $\log k'$ *versus* φ. Here the hypothetical curve for $A\varphi^2$ is shown with filled squares. Similarly, filled diamonds are used for the hypothetical effect of $B\varphi + C$. The sum of the $A\varphi^2$ and the $B\varphi + C$ curves is represented by the triangles.

Many studies have suggested or demonstrated that, over a limited range, retention of solutes in aqueous/organic mobile phase systems can be approximated by the relation

$$\log k' = \log k'_w - S\,\varphi \qquad (2)$$

where k'_w is the capacity factor for a solute in pure water and S represents the strength of the organic mobile phase component [2,3,5,15,16]. In some studies, the linear approximation extended to nearly 70% of the entire range of eluent composition for the methanol/water

system [10,15]. Cheong and Carr [10] have provided an excellent discussion of the limitations of empirical single-parameter scales. They contend that linear correlation of such scales is limited to a very narrow range. The results of the present study affirm this in that plots of log k' *versus* φ show quadratic behavior over nearly the entire range of solvent composition. This can be seen in Fig. 6 where the effect of the B term is convergent with the sum of the A, B, and C effects only at very low values of φ.

Conclusions

A quadratic dependence was found for plots of log k' *versus* φ in three nonaqueous binary mobile phase systems for several solutes. The use of nonaqueous mixtures on a polystyrene-divinylbenzene column allows direct measurement of k'_0 as well as the solute capacity factor over the entire range of mobile phase composition. With hexane as the weak solvent in the binary mixture, there is a steady decrease in retention with an increase in the polarity of the mobile phase. In this case, the system behaved like normal-phase chromatography, even though a non-polar stationary phase was used. This is evidenced by *p*-cresol having longer retention than very large PAH compounds. From this result it is expected that a polar functional group on the PS-DVB surface could influence retention of polar compounds in the presence of traditional normal-phase solvents. In the present case, we see that a polystyrene-divinylbenzene column can be used for reversed-phase and normal-phase separations.

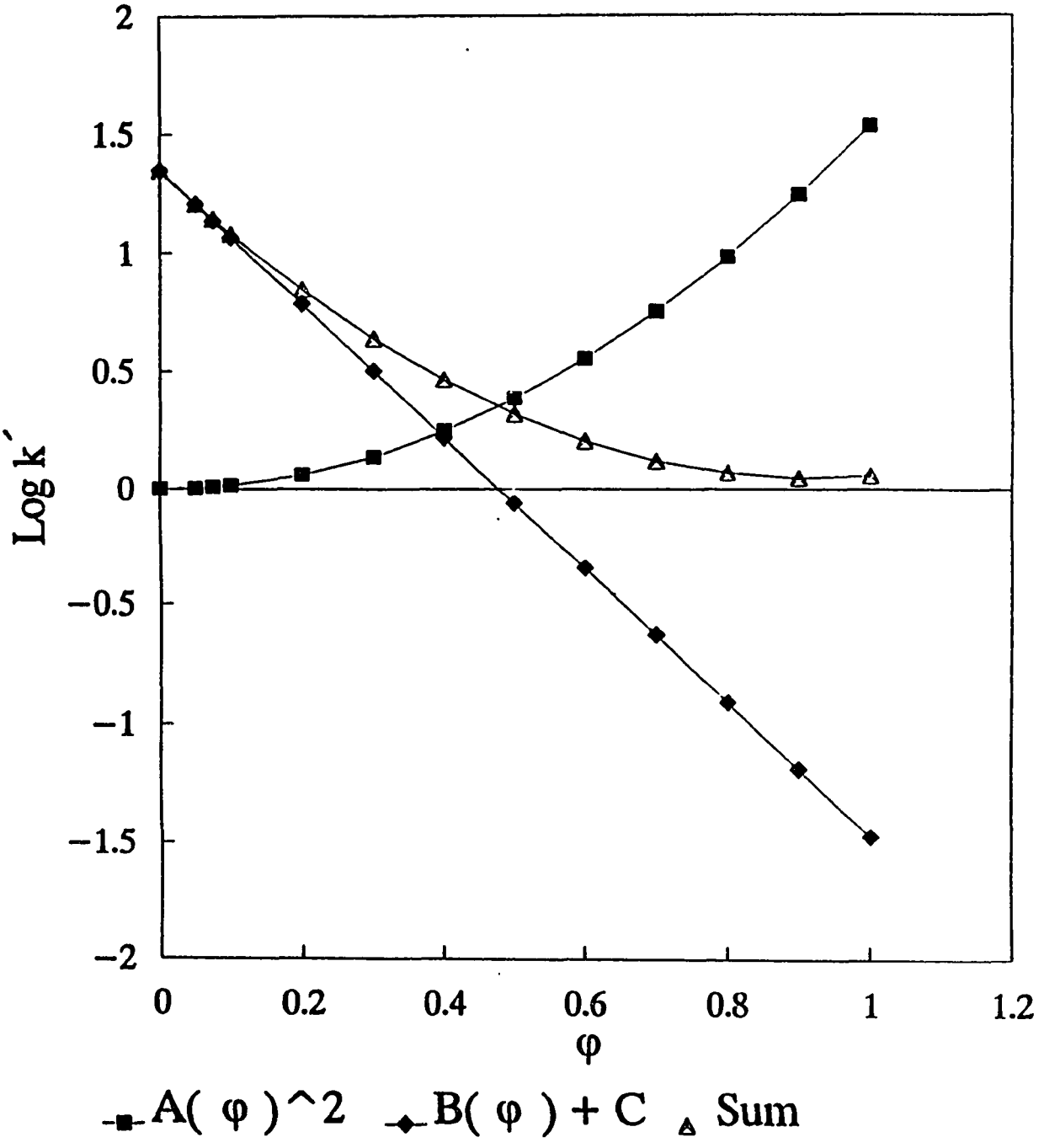

Fig. 6. Hypothetical plot showing the effect of parameters *A*, *B*, and *C*. Experimental values for *A*, *B*, and *C* from anthracene in methanol/ethyl acetate were used.

Acknowledgements

We thank Doug Gjerde of Sarasep, Inc. for resins used in this research. This work was supported by the Office of Basic Energy Sciences, U.S. Department of Energy and in part by a grant from the 3M Co., St. Paul, MN. The work was performed at the Ames Laboratory at Iowa State University. Ames Laboratory is operated for the U.S. Department of Energy under Contract No. W-7405-Eng-82.

References

[1] T.K. Chambers and J.S. Fritz, *J. Chromatogr. A*, in press.

[2] L.R. Snyder, J.W. Dolan, and J.R. Gant, *J. Chromatogr.*, 165 (1979) 3.

[3] J.W. Dolan, J.R. Gant, and L.R. Snyder, *J. Chromatogr.*, 165 (1979) 31.

[4] P.J. Schoenmakers, *Optimization of Chromatographic Selectivity*, Elsevier, Amsterdam, 1986, pp. 62.

[5] P.P. Csokán, F. Darvas, F. Csizmadia, and K. Valkó, *LC·GC*, 12, No. 1 (1994) 40.

[6] B.L. Karger, J.R. Gant, A. Hartkopf, and P.H. Weiner, *J. Chromatogr.*, 128 (1976) 65.

[7] J.W. Dolan, *LC·GC*, 13, No. 1 (1995) 24.

[8] P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen, and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.

[9] P.J. Schoenmakers, H.A.H. Billiet, and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.

[10] W.J. Cheong and P.W. Carr, *Anal. Chem.*, 61 (1989) 1524.

[11] L.C. Tan and P.W. Carr, *J. Chromatogr. A*, 656 (1993) 521.

[12] M.J. Kamlet, R.M. Doherty, and R.W. Taft, *J. Chem. Soc., Perkin Trans.*, II (1988) 2087.

[13] P.C. Sadek, P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, and M.H. Abraham, *Anal. Chem.*, 57 (1985) 2971.

[14] P.W. Carr, R.M. Doherty, M.J. Kamlet, R.W. Taft, W. Melander, and Cs. Horváth, *Anal. Chem.*, 58 (1986) 2674.

[15] K. Karch, I. Sebastian, I. Halász, and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.

[16] N. Sadlej-Sosnowska, I. Śledzińska, *J. Chromatogr.*, 595 (1992) 53.

AN EQUILIBRIUM-BASED METHOD FOR STUDYING THE EFFECT OF SOLVENT COMPOSITION ON SOLUTE CAPACITY FACTOR

A paper submitted to *Journal of Chromatography*

Thomas K. Chambers and James S. Fritz

Abstract

A method based on the equilibrium between solute and solvent is proposed for the interpretation of retention data in HPLC. Plots representing changes in retention as a function of mobile-phase composition showed linear dependence for nearly the entire range of solvent composition for nonaqueous binary systems. For methanol/water solvent systems, the interaction between the solute and unassociated methanol was considered. A data set from the literature was examined. Plots of retention *versus* molar concentration of methanol unassociated with water showed acceptable linearity over almost the entire range of eluent composition. The slope of the graph was interpreted to be the average combining ratio of solvent molecules to solute molecules. The results from the methanol/water system lend chromatographic support to the hypothesis of Scott *et al.* that a 1:1 methanol/water associate exists in methanol/water mobile phase mixtures.

Introduction

Several models have been presented to describe the dependence of solute retention on mobile phase composition [1-5]. The relationship between the log of the capacity factor (k') and the volume fraction of organic modifier (φ) in an aqueous/organic mobile phase has received much attention. In such systems, the mobile phase solvent composition needs to be

adjusted so that the capacity factors of the sample solutes are in a suitable range for an effective separation. A range of 1 to 10 has often been considered ideal for reasonable k' values. By knowing the relationship between k' and φ, solute retention can be predicted. Diagrams of some function of k' against φ are helpful in finding conditions where the separation factor for two solutes is the greatest. Finally, extrapolation of a plot of some function of k' *versus* φ can be useful in estimating the value of the capacity factor of a solute in pure water, k'_w. Often k'_w is too large for direct experimental measurement. Many investigations have examined the purported linear dependence of retention on mobile phase composition [1,3,6] described by

$$\log k' = \log k'_w - S\varphi \qquad (1)$$

where k' is the capacity factor of a solute at a given mobile phase composition, k'_w is the capacity factor for a compound extrapolated to zero percent organic modifier, S is the slope of the linear regression of the plot of log (or ln) k' *versus* φ. According to Sadlej-Sosnowska and Śledzińska, many interpretations of chromatographic data using this approach have shown linear correlations [7].

However, more careful investigation has shown that a plot of log k' against φ is linear only over a limited range of φ values. The correct relationship between log k' and mobile-phase composition has been shown to be quadratic in nature [8-10]. Schoenmakers and co-workers reported the following equation:

$$\ln k' = A\varphi^2 + B\varphi + C \qquad (2)$$

where A,B,and C are empirical constants [8,9]. The B term usually has a negative value that is sufficiently large for the entire plot to slope downward as φ increases. For binary solvent systems, the curve can often be approximated by a straight line over a restricted segment of the plot [11]. It frequently happens that the pseudo-linear segment corresponds to the region where k' is between approximately 1 and 10.

The slope S in Eq. (1) is defined as the solvent strength of the organic modifier and was at first considered to be independent of solute size or structure [1,3]. This would hypothetically lead to plots of log k' *versus* log φ with equal slopes. The S value has been used in some discussions to compare strengths of different organic solvents on a semi-quantitative basis, irrespective of solute type [12-15]. Originally S was considered to be a constant. Poole & Poole show a series of solvent strengths as methanol < acetonitrile < acetone < dioxane < ethanol < isopropanol < tetrahydrofuran [12]. A similar eluotropic series is given by Engelhardt and co-workers [3] where plots of k' *versus* φ were shown to be linear for 0 to 50% methanol. However, this picture of solvent strength (S) seems far too simplistic. Chen *et al.* [13-15] reported that S is a variable that tends to increase with increasing solute retention (higher k' values). There is a general trend for S to increase as the molecular size (and approximately molecular weight) of the solute becomes larger [11,16,17]. S also increases in the series: aniline < alkylbenzene < chlorobenzene < ether < aldehydes, ketones < nitriles < unsubstituted polyaromatics < nitro compounds < phthalates < phenylalkanols, according to Chen *et al.* H-bonding results in a dramatic decrease in S. In summary, polar

compounds have lower S and larger compounds have larger S. Thus, S is a solute-related constant. In Eq. (1), the mobile phase effects determine slope, the stationary phase effects determine the intercept, and solute molecular structure influences both [13].

Solvatochromic measurements may give a better insight than simple solvent composition into solvent effects and their effect on capacity factor. Certain dyes have considerably different absorption wavelengths in different solvent mixtures. The $E_{T(30)}$ scale of solvent strength is based on the absorption of 2,6-diphenyl-4-(2,4,6-triphenyl-*N*-pyridinio)phenolate in solvents of different polarity. The shift in absorbance caused by dissolution of this compound in different solvents is a measure of the dipolarity of the solvents. Here solvent effects are measured apart from stationary phase interactions. Plots of log k' *versus* $E_{T(30)}$ have in some cases shown better linearity than plots of log k' *versus* φ [18,19].

The use of concepts such as S and $E_{T(30)}$ has been studied in detail by W.J. Cheong and P.W. Carr [19]. They concluded that all empirical single-parameter solvent strength scales have severe limitations. One weakness in single-parameter scales lies in the possible co-variance of two parameters over a limited segment of the graph, thus creating the appearance of single-parameter dependence. For example, a given correlation may appear to be a function of solute size or structure alone when it may actually include the effect of solute-solvent interactions that affect k' to a similar extent as solvent composition is varied.

In the present work, a simple equilibrium model is used to describe the effect of a binary mobile phase composition on the capacity factor (k') of a sample solute (A). The mobile phase can be a mixture of water and an organic solvent or a mixture of two organic

solvents. The decrease in k' with an increase in the fraction (φ) of the stronger solvent in the mobile phase is a result of stronger solvation of A by that solvent. An equilibrium is assumed to exist in which some fraction of A is associated with the stronger mobile phase solvent ($A\varphi$), while the rest remains as free A (or A that is solvated only by the weaker solvent). If we define R as the ratio of $A\varphi$: A in solution, it is shown that log R is a linear function of log φ.

Experimental

Two data sets were examined. First, the data from Chambers & Fritz [22] were used to calculate values for log R as a function of the volume fraction of the strong solvent in the binary eluent. Regression for the plots was calculated for the best fit line to the data. For the second set, the data reported in the work of Schoenmakers *et al.* [9] was examined for the methanol/water solvent system. Constants given for A, B, and C were used in equation (2) to calculate values for capacity factor. It should be noted that Schoenmakers and co-workers collected data for solvent mixtures from 10-100% organic modifier with column temperature thermostated at 25° C. We calculated the molar concentration of unassociated methanol from the values reported by Scott and co-workers [21] for percent (v/v) of unassociated methanol in a methanol/water mixture. Plots of log R vs. log (molar conc. MeOH) were generated and the regression was calculated for the best fit line for each of 31 compounds reported by Schoenmakers *et al.* for the methanol/water system.

Results and Discussion

Theory

Suppose a solute (A) is dissolved in water and the solution is equilibrated with a hydrophobic chromatographic stationary phase. The capacity factor (k'_w) describes the partitioning if the solute between the two phases. Most organic solutes will partition strongly into the stationary phase and the value of k'_w is apt to be quite high, often a value of several hundred or even several thousand. If the same solute is now dissolved in a pure organic solvent and the solution is equilibrated with the same chromatographic stationary phase, the solute will be strongly solvated by the liquid solvent and the value of the capacity factor k'_{org} will be quite small, perhaps approaching zero. In any mixture of water and the organic solvent, the capacity factor will decrease as the fraction of the organic solvent in the liquid mixture is increased.

Solvation of the organic solute by the organic solvent will generally be much stronger than solvation by water, as evidenced by the large difference in capacity factors in going from pure water to pure organic solvent. This large difference in solvation of the solute by water and the organic solvent permits the use of an equilibrium approach:

$$A + nL \rightleftharpoons AL_n \qquad (3)$$

where A is the molar concentration of free, unsolvated solute in solution, L is the molar concentration of organic solvent in the aqueous/organic solvent mixture, n is the number of molecules of organic solvent associated with a single solute molecule, and AL_n is the molar concentration of the solvated solute in solution. The equilibrium constant for this expression

is

$$K = \frac{AL_n}{A \cdot L^n} \qquad (4)$$

If the ratio of $AL_n : A$ is defined as R, then

$$R = KL^n \qquad (5)$$

If the value of k'_w is very large compared to k'_{org}, the capacity factor for the solute in any given water-organic mixture (k')will depend almost entirely on the fraction of unassociated solute in the equilibrium mixture, α_A,

$$k' = \alpha_A \, k'_w \qquad (6)$$

$$\alpha_A = \frac{A}{A + AL_n} \qquad (7)$$

Combining equations 6 and 7, then inverting

$$\frac{k'_w}{k'} = 1 + \frac{AL_n}{A} = 1 + R \qquad (8)$$

$$R = \frac{k'_w}{k'} - 1 \qquad (9)$$

Thus R can be evaluated simply by measuring k'_w and k'. By converting equation 3.5 to logarithms and using experimental k'_w and k' values, the following can be used:

$$\log R = n \log L + \log K \qquad (10)$$

A plot of log R *versus* log L is expected to be linear with a slope of n. When log R = 0, the log K is equal to the quantity -$n$log L.

Hexane/1-chlorobutane system

In a recent paper, we investigated the chromatographic behavior of several analytes using a mixture of two organic solvents as the mobile phase using a polystyrene-divinylbenzene column [22]. The capacity factors were measured over the entire range of φ = 0 to φ = 1, where φ is the volume fraction of the solvent with the stronger solvating properties for the analytes. For example, in a binary mixture of hexane and 1-chlorobutane (CB), the CB has the stronger solvating ability. In the binary nonaqueous systems studied, it was possible to measure the capacity factors at φ = 0. In water/organic systems, the capacity factors are often too high to be measured experimentally at low values of φ.

Data from that work was used to demonstrate the utility of plotting with the model presented here. Retention data for chrysene in the hexane/1-chlorobutane mobile phase system are given in Table 1. A curve-fitting technique showed that a plot of log k' *versus*

φ follows a quadratic dependence with a correlation coefficient of 0.999 at the 95% confidence level. If the same data for chrysene are plotted using log *R versus* log *L* (the total molar concentration of 1-chlorobutane), the result is a linear correlation with a coefficient of 0.999. The graph of log *R versus* log *L* for chrysene in hexane/1-chlorobutane is shown in Fig 1. The plot is linear from 10% to 100% volume fraction of 1-chlorobutane.

Similar results were obtained for many of the solutes from reference 22. In the methanol/ethyl acetate system, the data point for chrysene in 100% methanol had to be estimated because of prohibitively long retention. The value for k' in 100% methanol was

Table 1
Retention data as a function of volume fraction 1-chlorobutane in nonaqueous hexane/1-chlorobutane mobile phase mixture

Solute	φ	*L*	*k'*	*R*
Chrysene	0.0	0.0	13.71	0.00
	0.1	0.95	7.74	0.77
	0.2	1.90	5.06	2.71
	0.3	2.86	3.56	2.85
	0.4	3.81	2.71	4.06
	0.5	4.76	2.19	5.26
	0.7	6.66	1.51	8.08
	0.9	8.57	1.23	10.15
	1.0	9.52	1.13	11.13

extrapolated from a curve fit to a plot of log k' *versus* φ. Table 2 lists the correlation results for plots of log R *versus* log L for several compounds in three nonaqueous binary mobile phase systems taken from reference 22.

Methanol/water system

Schoenmakers, Billiet, and de Galan published data for 31 solutes in methanol/water in which log k' was shown to be a quadratic function of the fraction of methanol (φ) in the solution phase [9]. From the constants given, the capacity factor of each of the solutes studied can be calculated over the entire range of solvent composition. Plotting these data by the same method used for the binary non-aqueous systems gave correlations that were less than satisfactory. For example, when the data for benzene were plotted as log R *versus* the log of L_T, where L_T is the *total* molar concentration of methanol (the concentration analog of φ), the resulting curve was clearly quadratic, as depicted in the lower trace of Fig. 2. A quadratic fit of the form $\log R = AL_T^2 + BL_T + C$ gave values of 1.3, 0.1, and -0.41 for A, B, and C, respectively, with a correlation coefficient of 0.998 at a 95 % confidence level. When log R *versus* log φ (volume fraction total methanol) was plotted for the same system, the graph was identical in shape but differed from the graph using log L_T by a constant.

Scott and co-workers found that a significant amount of a methanol/water complex (MW) is present in methanol/water mixtures [20,21]. They determined the equilibrium constant for the methanol/water complex and gave a table of the fraction of M, W and MW present in methanol/water mixtures. Scott hypothesized but did not prove chromatographically that the capacity factor of a solute depends on the free, unassociated

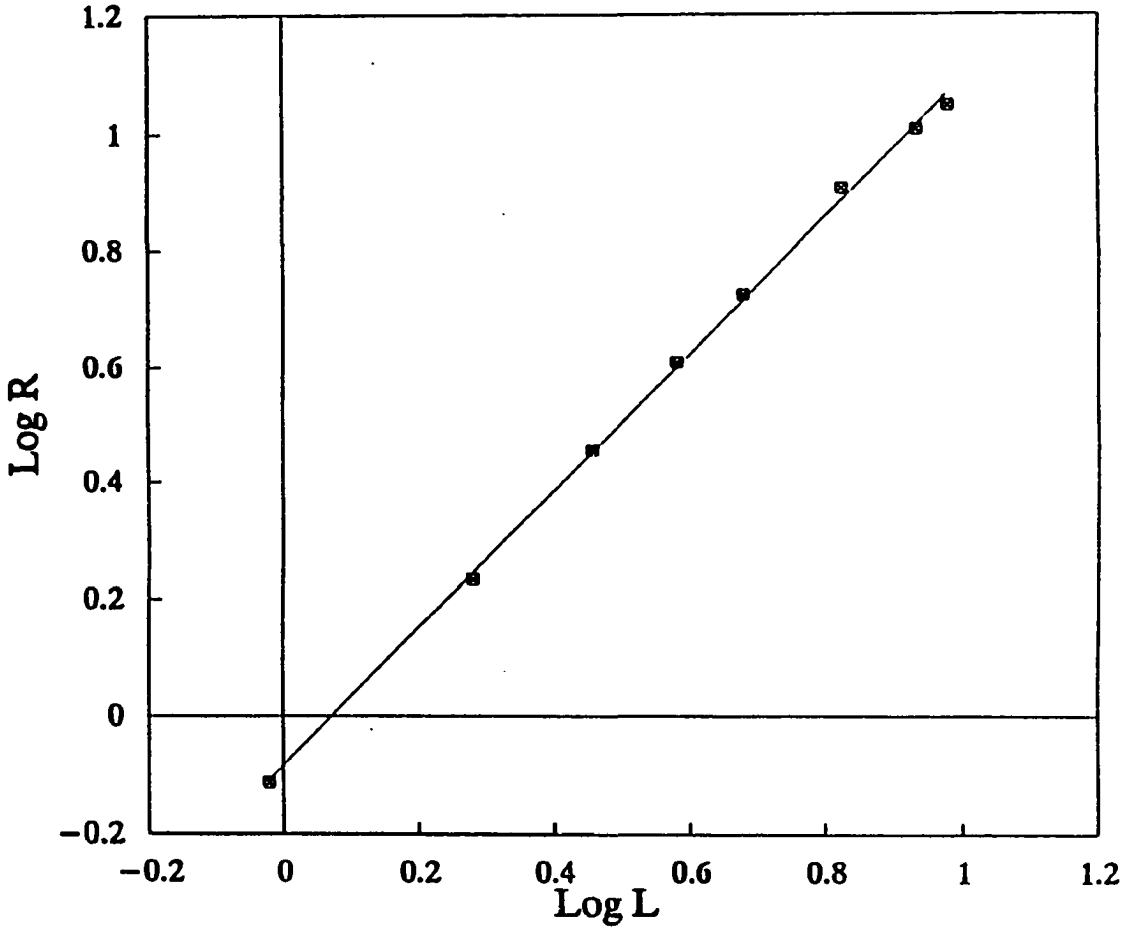

Fig. 1. Log *R versus* log *L* for chrysene in hexane/1-chlorobutane on a 10 cm, 5 μm d_p polystyrene-divinylbenzene column. UV detection at 254 nm. Flow rate: 1 mL/min.

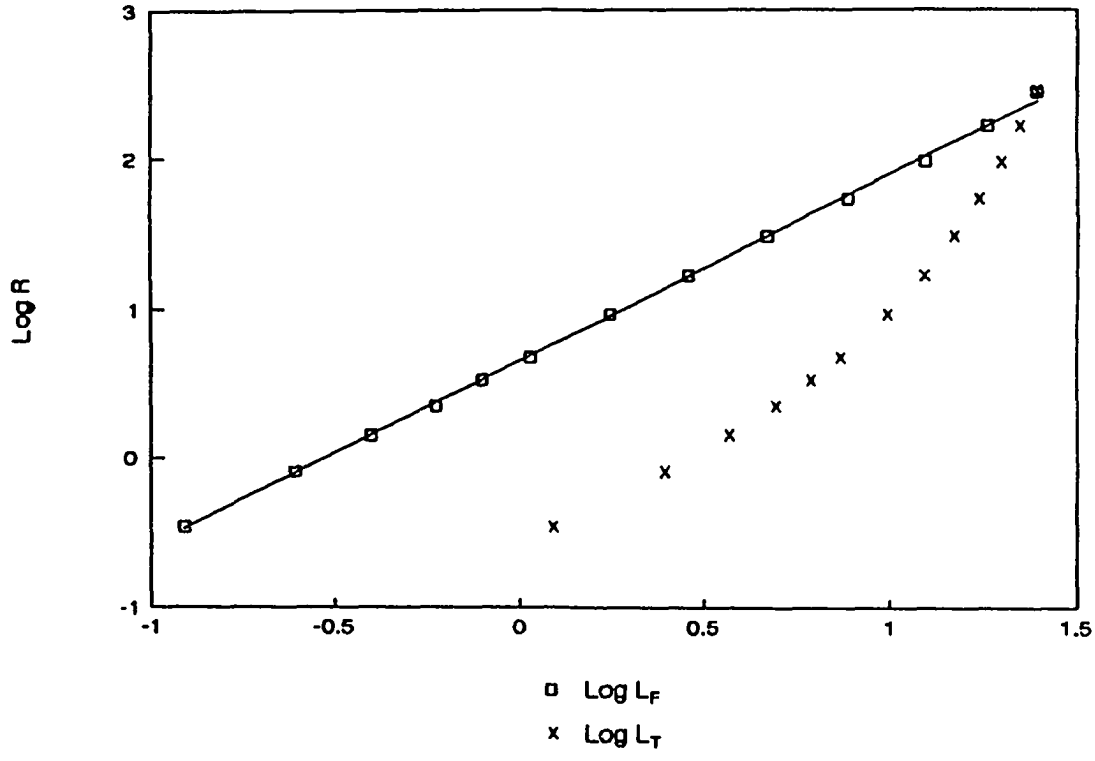

Fig. 2. Log R *versus* log L_F and log R *versus* log L_T for benzene in methanol/water on a bonded-phase silica column. Data taken from reference 9.

Table 2
Correlation results for log *R versus* log *L* for three nonaqueous binary mobile phase systems

Mobile Phase Mixture	Solute	*n*	S.D.	*K*	S.D.	Correlation Coefficient
Acetonitrile/Ethyl Acetate	Naphthalene	0.70	0.02	0.40	0.02	0.990
	Anthracene	0.95	0.02	0.51	0.02	0.995
	Chrysene	1.14	0.03	0.59	0.02	0.996
Methanol/Ethyl Acetate	Anthracene	1.23	0.03	1.68	0.03	0.996
	Chrysene	1.48	0.05	3.10	0.04	0.992
Hexane/1-Chlorobutane	Toluene	0.59	0.02	0.22	0.02	0.987
	Naphthalene	0.83	0.02	0.33	0.02	0.996
	Anthracene	0.93	0.04	0.62	0.04	0.986
	Chrysene	1.18	0.02	0.82	0.01	0.999
	Phenethyl Alcohol	1.01	0.01	0.85	0.01	0.9998
	p-Cresol	1.29	0.01	0.82	0.01	0.9995

methanol rather than the total methanol in the mobile phase.

The data from Schoenmakers [9] were used to plot log R against the log of the molar concentration of free methanol, rather than the total methanol concentration. The plot for benzene is given in the upper graph in Fig. 2. This plot is linear over a broad range of free L values with a correlation coefficient of 0.999. Linear plots were obtained for all of the 31 compounds studied when the free methanol concentration was used. The values for the slopes, intercepts, and correlation coefficients are listed in Table 3. Excellent linear correlations were obtained for plots of log R *versus* log of the free methanol concentration over nearly the entire range of solvent composition. The error in measuring R increases when the value of R is either very small of very large.

These results strongly support the hypothesis by Scott *et al.* that a methanol/water association complex affects HPLC retention when a methanol/water mobile phase is used. To our knowledge, this is the first chromatographic test of this hypothesis. It seems reasonable to assume that methanol and water could form strongly hydrogen-bonded association complexes. In the non-aqueous binary systems studied, however, solvent-solvent interactions would be limited to weak van der Waals and dispersive forces, making it difficult for associations to form to a degree that would be significant in chromatography.

The slope n of the graphs of log R *versus* log L_F deserves comment. n is the combining ratio of analyte to strong solvent ligand, in this case unassociated methanol. It might be expected that n would be restricted to integer values. In solution, however, we can infer that there will be an average value of integer combinations of solute and strong solvent molecules that depend on the size of the solute with respect to that of the solvent molecule

and on solute-solvent interactions. Accordingly, the equilibrium constant K includes the step-wise formation constants for the association of increasing numbers of the ligand L with solute A. The possibility of interactions between water and the solute molecules can be neglected based on the extreme retention of the solute in 100% water.

Table 4 lists the values for k'_w, the slope of the graph (n), and the antilog of the intercept (K) by order of increasing value of n. It can be seen here that the value of k'_w generally increases with increasing n. When the logarithm of k'_w was plotted as a function of n, a nearly linear plot was obtained with a slope of 2.92 ± 0.04 and an intercept of -1.47 ± 0.08 and is shown in Fig. 3. The value of the correlation coefficient is 0.995 showing acceptable dependence of k'_w on n for all 31 compounds in the Schoenmakers *et al.* data set [9].

Conclusions

A simple equilibrium model serves to correlate chromatographic retention of analytes in HPLC with mobile-phase composition. Plots of log R *versus* the log of the concentration of the stronger solvent showed linear dependence for analytes tested in three non-aqueous binary systems. Poor correlations were observed for 31 analytes in methanol/water systems when the concentration of total methanol was used. However, excellent linear plots were obtained for log R *versus* log free methanol concentration.

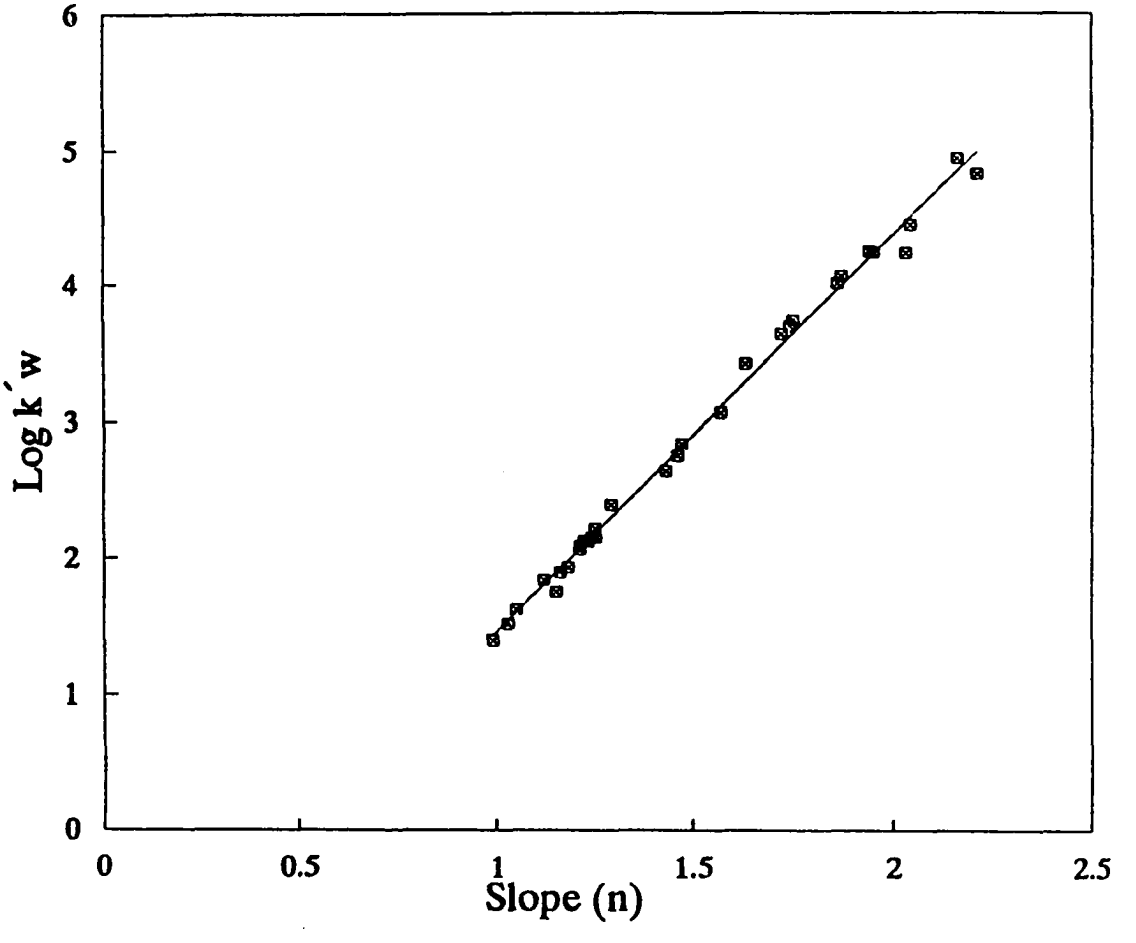

Fig. 3. Log k'_w *versus* n for 31 compounds from reference 9.

Table 3
Correlation results for log *R versus* log *L* for the methanol/water system. Data taken from reference 9.

Solute	*n*	S.D.	*K*	S.D.	Correlation Coefficient
Acetophenone	1.29	0.03	11.26	0.08	0.993
Anethole	1.87	0.03	25.36	0.08	0.997
Aniline	0.98	0.03	3.55	0.07	0.992
Anisole	1.95	0.07	99.62	0.2	0.987
Benzaldehyde	1.12	0.02	5.05	0.05	0.997
Benzene	1.25	0.01	4.52	0.03	0.999
Benzonitrile	1.21	0.03	8.06	0.07	0.990
Benzophenone	2.21	0.05	135.02	0.1	0.995
Benzyl Alcohol	1.05	0.03	5.01	0.08	0.991
Biphenyl	1.94	0.04	26.54	0.1	0.996
Chlorobenzene	1.75	0.02	27.98	0.05	0.999
o-Cresol	1.25	0.02	7.68	0.05	0.997
Diethyl-*o*-phthalate	2.03	0.05	92.98	0.1	0.993
N,N-Dimethylaniline	1.72	0.02	26.85	0.05	0.999
2.4-Dimethylphenol	1.46	0.02	13.16	0.04	0.999
Dimethyl-*o*-phthalate	1.57	0.05	33.59	0.1	0.988
m-Dinitrobenzene	1.16	0.01	3.31	0.02	0.999
Diphenyl Ether	2.04	0.03	40.06	0.09	0.997
Ethylbenzene	1.75	0.02	20.43	0.06	0.998
N-Methylaniline	1.47	0.03	17.15	0.07	0.996
Naphthalene	2.16	0.06	33.29	0.2	0.990
p-Nitroacetophenone	1.23	0.02	7.23	0.05	0.997
o-Nitroaniline	1.16	0.02	5.72	0.05	0.997
Nitrobenzene	1.24	0.01	6.00	0.02	0.999
m-Nitrophenol	1.18	0.02	6.22	0.05	0.997

Phenol	1.03	0.03	4.29	0.07	0.992
1-Phenylethanol	1.22	0.03	8.71	0.07	0.994
2-Phenylethanol	1.21	0.03	7.87	0.07	0.995
3-Phenylpropanol	1.43	0.02	13.41	0.05	0.998
Quinolone	1.86	0.06	78.08	0.2	0.987
Toluene	1.63	0.02	18.99	0.04	0.999

Table 4
Values for retention in 0% strong solvent, slope, and intercept (antilog) for plots of log *R* *versus* log *L* for solutes in the Schoenmakers *et al.* data set (reference 9).

Solute	k'_w	*n*	*K*
Aniline	24	0.99	3.6
Phenol	32	1.03	4.3
Benzyl Alcohol	41	1.05	5.0
Benzaldehyde	67	1.12	5.0
m-Dinitrobenzene	55	1.15	3.3
o-Nitroaniline	77	1.16	5.7
m-Nitrophenol	84	1.18	6.2
2-Phenylethanol	114	1.21	7.9
Benzonitrile	120	1.21	8.1
1-Phenylethanol	133	1.22	8.7
p-Nitroacetophenone	130	1.23	7.2
Nitrobenzene	140	1.24	6.0
o-Cresol	141	1.25	7.7
Benzene	162	1.25	4.5
Acetophenone	247	1.29	11.3
3-Phenylpropanol	441	1.43	13.4
2,4-Dimethylphenol	572	1.46	13.2
N-Methylaniline	692	1.47	17.2
Dimethyl-*o*-phthalate	1180	1.57	33.6
Toluene	2620	1.63	19.0
N,N-Dimethylaniline	4320	1.72	26.9
Cholrobenzene	4870	1.74	28.0
Ethylbenzene	5430	1.75	20.4
Quinolone	10200	1.86	78.1
Anethole	11500	1.87	25.4
Biphenyl	17500	1.94	26.5

Anisole	17200	1.95	99.6
Diethyl-*o*-phthalate	17000	2.03	93.0
Diphenyl Ether	27200	2.04	40.1
Naphthalene	85800	2.16	33.3
Benzophenone	65500	2.21	135.0

Acknowledgements

We extend thanks to Doug Gjerde of Sarasep for the polystyrene-divinylbenzene material used in this research. This work was supported by the Office of Basic Energy Sciences, U.S. Department of Energy, and in part by a grant from 3M (St. Paul, MN, USA). This study was undertaken at the Ames Laboratory at Iowa State University. Ames Laboratory is operated for the U.S. Dept. of Energy under Contract No. W-7405-Eng-82.

References

[1] L.R. Snyder, J.W. Dolan, and J.R. Gant, *J. Chromatogr.*, 165 (1979) 3.

[2] J.W. Dolan, J.R. Gant, and L.R. Snyder, *J. Chromatogr.*, 165 (1979) 31.

[3] K. Karch, I. Sebastian, I. Halász, and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.

[4] R. Tijssen, H.A.H. Billiet, and P.J. Schoenmakers, *J. Chromatogr.*, 122 (1976) 185.

[5] J.H. Hildebrand and R.L. Scott, *The Solubility of Non-Electrolytes*, Dover, New York, 1964.

[6] P.P. Csokán, F. Darvas. F. Csizmadia, and K. Valkó, *LC•GC*, 12 (1) (1994) 40.

[7] N. Sadlej-Sosnowska and I. Śledzińska, *J. Chromatogr.*, 595 (1992) 53.

[8] P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen, and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.

[9] P.J. Schoenmakers, H.A.H. Billiet and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.

[10] L.C. Tan and P.W. Carr, *J. Chromatogr. A*, 656 (1993) 521.

[11] M.A. Quarry, R.O. Grob, L.E. Snyder, J.W. Dolan, and M.P. Rigney, *J. Chromatogr.*, 384 (1987) 163.

[12] C.F. Poole and S.K. Poole, *Chromatography Today*, Elsevier, Amsterdam, 1991.

[13] N. Chen, Y. Zhang, and P. Lu, *J. Chromatogr.*, 633 (1993) 31.

[14] N. Chen, Y. Zhang, and P. Lu, *J. Chromatogr.*, 606 (1992) 1.

[15] N. Chen, Y. Zhang, and P. Lu, *J. Chromatogr.*, 603 (1992) 35.

[16] L.R. Snyder, M.A. Quarry, and J.L. Glajch, *Chromatographia*, 24 (1987) 33.

[17] X. Geng and F.E. Regnier, *J. Chromatogr*, 332 (1985) 147.

[18] B.P. Johnson, M.G. Khaledi, and J.G. Dorsey, *Anal. Chem.*, 58 (1986) 2354.

[19] W. J. Cheong and P.W. Carr, *Anal. Chem.*, 61 (1989) 1524.

[20] E.D. Katz, K. Oagan, and R.P.W. Scott, *J. Chromatogr.*, 352 (1986) 67.

[21] E.D. Katz, C.H. Lochmüller, and R.P.W. Scott, *Anal. Chem.*, 61 (1989) 349.

[22] T. K. Chambers and J.S. Fritz, High Performance Liquid Chromatography with Nonaqueous Binary Solvent Mixtures, in preparation.

THE EFFECT OF POLYSTYRENE-DIVINYLBENZENE COLUMN SULFONATION ON SOLUTE RETENTION IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

A paper to be submitted to *Chromatographia*

Thomas K. Chambers and James S. Fritz

Abstract

A series of columns of increasing sulfonation capacity were used to study the effect of polystyrene-divinylbenzene sulfonation on solute retention in conventional reversed-phase and normal-phase eluents. Solutes chromatographed using a reversed-phase eluent showed a decrease in retention as sulfonation capacity was increased. In the presence of a predominantly organic mobile phase, polar compounds were retained longer with an increase in sulfonation capacity. Plots of log *k' versus* φ for polar compounds separated on a column of relatively high sulfonation capacity in 95% acetonitrile had positive slopes, the opposite of what is typically encountered when reversed-phase solvents are used. These curves, however, were more accurately described by quadratic fit. Linearity was achieved when an equilibrium model was applied for the correlation of the data. Graphs of *k' versus* sulfonation capacity increased smoothly for solutes separated on a range of sulfonated polystyrene-divinylbenzene columns.

Introduction

We have shown in related work [1-3] the utility of polystyrene-divinylbenzene (PS-DVB) columns for the study of solute retention in high-performance liquid chromatography. These columns provide higher retention than conventional bonded-phase silica packings and

allow the separation of single- and multiple-ring compounds in the presence of 100% organic mobile phases. Such separations provide data that are useful for optimizing separations and allow measurement of chromatographic values such as the capacity factor in 100% weak solvent, which has shown to be elusive in traditional reversed-phase conditions.

Bonded-phase silica packings such as octadecylsilane (ODS) have gained broad acceptance as the material of choice for reversed-phase separations of low molecular weight organic molecules, providing short analysis times and high column efficiency. These materials are not stable, however, under conditions of extreme pH and may produce peak tailing for basic solutes. Polystyrene-divinylbenzene columns have proven useful for many separations including reversed-phase, ion-exclusion, solid-phase extraction, and anion exchange and provide a worthy alternative to ODS packings [1-8].

During the development of ion chromatography, sulfonated polystyrene-divinylbenzene stationary phases quickly became the most popular type of cation exchange material [9,10]. Sevenich and Fritz used sulfonated PS-DVB for the separation of Mn^{2+}, Mg^{2+}, alkaline earths, and several other metal cations by ion chromatography [11,12]. These papers presented the results of experiments using low-capacity (6.1 μeq/g) ion exchangers instead of high-capacity materials popular in early ion chromatography studies. Linear plots were obtained for log adjusted retention time *versus* log eluent ion activity. A paper by the same authors reported conditions for the preparation of sulfonated PS-DVB beads for ion chromatography [13]. That work showed that sulfonation occurred on the surface of the 12-17 μm, 12 % cross-linked beads.

Schmidt and Fritz used low exchange capacity sulfonated PS-DVB material to

preconcentrate organic solutes from aqueous matrices, followed by group separation on the same material [14]. Morris and Fritz compared carboxylated polyacrylate and sulfonated PS-DVB packings for the separation of organic acids and small polar compounds by ion-exclusion [15]. They concluded that the mechanism of retention was a partitioning of solutes between the mobile and stationary phases.

Sun used sulfonated PS-DVB stationary phases in HPLC [9]. These investigations showed that sulfonated PS-DVB packings could be used for separating basic compounds from neutral compounds and weakly basic amines using a pre-column. A range of sulfonation capacities was used in an on-line mode to group-separate basic compounds before further separation on a porous PS-DVB HPLC column. Sulfonated polystyrenes were also used to separate basic and neutral compounds. Plots of log *k' versus* sulfonation capacity showed linear correlations for several compounds on a 10 cm x 4.6 mm column packed with 10 μm sulfonated PS-DVB.

Dumont, Fritz, and Schmidt demonstrated the use of sulfonated macroporous PS-DVB packings for ion-exchange chromatography in non-aqueous solvents [15-17]. The results suggested that solvation of the stationary phase contributes significantly to analyte selectivity. The use of non-aqueous eluents allowed separations that are often difficult to achieve in aqueous mobile phases. Addition of 18-crown-6 to the eluent improved peak shape and resolution for many ions [15]. For cation-exchange of several amines, plots of log *k' versus* log H^+ had slopes of nearly -1, indicating a purely cation-exchange mechanism instead of a mixed-mode mechanism involving hydrophobic interactions [17].

Dumont and Fritz also used sulfonated PS-DVB material (8 μm) in solid-phase

extraction studies [6]. It was shown that surface sulfonation of PS-DVB solid-phase extraction (SPE) material facilitated contact between solutes dissolved in water and the surface of the SPE packing. The best recoveries for many polar compounds were found at a PS-DVB sulfonation capacity of 0.6 meq/g.

In the present study, we have undertaken to investigate the effect of the presence of sulfonic acid groups chemically bound to the surface of the polystyrene-divinylbenzene bead. Specifically, we observed the effect of PS-DVB sulfonation on the retention of polar and nonpolar solutes in primarily aqueous eluents and polar solutes in a chiefly organic mobile phase in HPLC separations. This was achieved by preparing a set of columns differing only in sulfonation capacity. A set of eight sulfonated PS-DVB columns ranging from 0 to 2.6 milliequivalents per gram was used. Each was of standard-bore HPLC column diameter (4.6 mm I.D.). A given set of analytes was chromatographed by HPLC on each of the sulfonated columns and the retention data were compared to observe the effect of column sulfonation on solute retention.

Experimental

Reagents and chemicals

Acetonitrile was obtained from Fisher Scientific (Pittsburgh, PA, USA) and was HPLC grade. Analyte compounds were purchased from Aldrich (Milwaukee, WI), Fisher, Mallinckrodt (St. Louis, MO, USA), or from Eastman Kodak (Rochester, NY, USA) and were of analytical reagent grade.

Apparatus

The chromatograph consisted of an Alltech model 425 HPLC pump (Deerfield, IL, USA), a model 783A UV absorbance detector (Applied Biosystems, Stone mountain, GA, USA), model 7000 switcher (Rheodyne, Cotati, CA, USA, used as injector), Hitachi model D-2000 *Chromato-Integrator* (EM Science, Cherry Hill, NJ, USA), and a model LP-21 *Lo-Pulse* pulse dampener (Scientific Systems Inc., State College, PA, USA). The columns were 4.6 mm I.D. x 10 cm stainless steel slurry-packed in our laboratory with bare or derivatized polystyrene-divinylbenzene (PS-DVB) beads of 10 μm average particle size and 80 Å to 100 Å average pore diameter (Sarasep, Santa Clara, CA, USA). An Eldex Laboratories column heater was used to maintain column temperature at 26^0 C.

Chromatographic procedure

Sample compounds were dissolved acetonitrile and water. Chromatographic eluents were sparged with helium (Air Products, Des Moines, IA, USA) for 5 min before transferring to the eluent reservoir. Binary aqueous/organic solvent mixtures were used as mobile phases at a flow rate of 1.2 mL/min. Samples were injected using a 5μL injection loop (Rheodyne, Cotati, CA, USA). Chromatographic peaks were detected using UV absorbance at 254 nm or 195 nm. Retention times were recorded with an integrator and the chromatographic capacity factor, k', was calculated for each analyte using the relation: $k' = (t_R - t_0)/t_0$. Column hold-up time was determined by measuring the retention time of bromide ion or nitrate ion. All chromatograms were generated isocratically and retention data were reported as the average of at least three injections.

Column sulfonation

The method of column sulfonation described by Dumont and Fritz was followed [6]. Briefly, underivatized bulk polystyrene-divinylbenzene particles (55% cross-linked) were slurried in acetic acid in an ice bath and then treated with concentrated sulfuric acid for a designated reaction time and subsequently poured over ice to quench the reaction. Reaction times depended on the surface concentration of sulfonic acid groups desired. Capacities were determined by titration according to the method described in reference 6. The sulfonated PS-DVB material was slurry-packed into stainless steel hardware at 3000 psi. Eight columns were used having capacities and reaction conditions as listed in Table 1.

Table 1
Reaction conditions for PS-DVB sulfonation

Capacity (mequiv./g)	H_2SO_4 (mL)	Reaction time	Temperature
0.00			
0.27	5	25 s	Ice
0.78	50	4 min	Ice
0.93	50	90 min	Room temperature
1.30	50	40 min	50^0 C
2.03	50	90 min	50^0 C
2.34	50	90 min	70^0 C
2.63	50	120 min	90^0 C

Results and Discussion

Effect of column sulfonation on the retention of solutes in a reversed-phase eluent

Typical reversed-phase eluents made up of 30% or 50% acetonitrile in water were used for the first segment of the study. These mixtures were chosen because of the expectation that retention times would become lower as progressively higher sulfonation capacities were used. Using 30% and 50% acetonitrile kept analysis times within a reasonable range. Fig. 1 shows a plot of *k' versus* sulfonation capacity for benzene in a 30% acetonitrile : 70% water mobile phase. We see a progressive, large decrease in retention as sulfonation capacity is increased. The graph is approximately linear. Deviation from linearity is probably due to errors in determining column sulfonation capacity by titration. The same trend is followed for four other aromatic compounds chromatographed using the same set of columns. Table 2 lists retention data as a function of sulfonation capacity for the five solutes tested. Chromatograms for these five compounds on three columns of increasing sulfonation capacity are compared in Fig. 2.

These results differ from those of Dumont and Fritz [18] where similar experiments were carried out in pure water. Results from their work showed a retention maximum at 0.6 milliequivalents per gram for plots of capacity factor *versus* sulfonation capacity for phenol and catechol. A maximum at 0.6 meq/g in pure water suggests that a mixed-mode retention mechanism exists. At zero sulfonation capacity, the surface of the particle is hydrophobic and the polar solutes are not retained in pure water. At 0.6 meq/g there are enough polar sulfonic acid groups on the surface to allow sufficient wetting of the surface by water, thus bringing the analyte into contact with the stationary phase. At higher sulfonation capacities, the

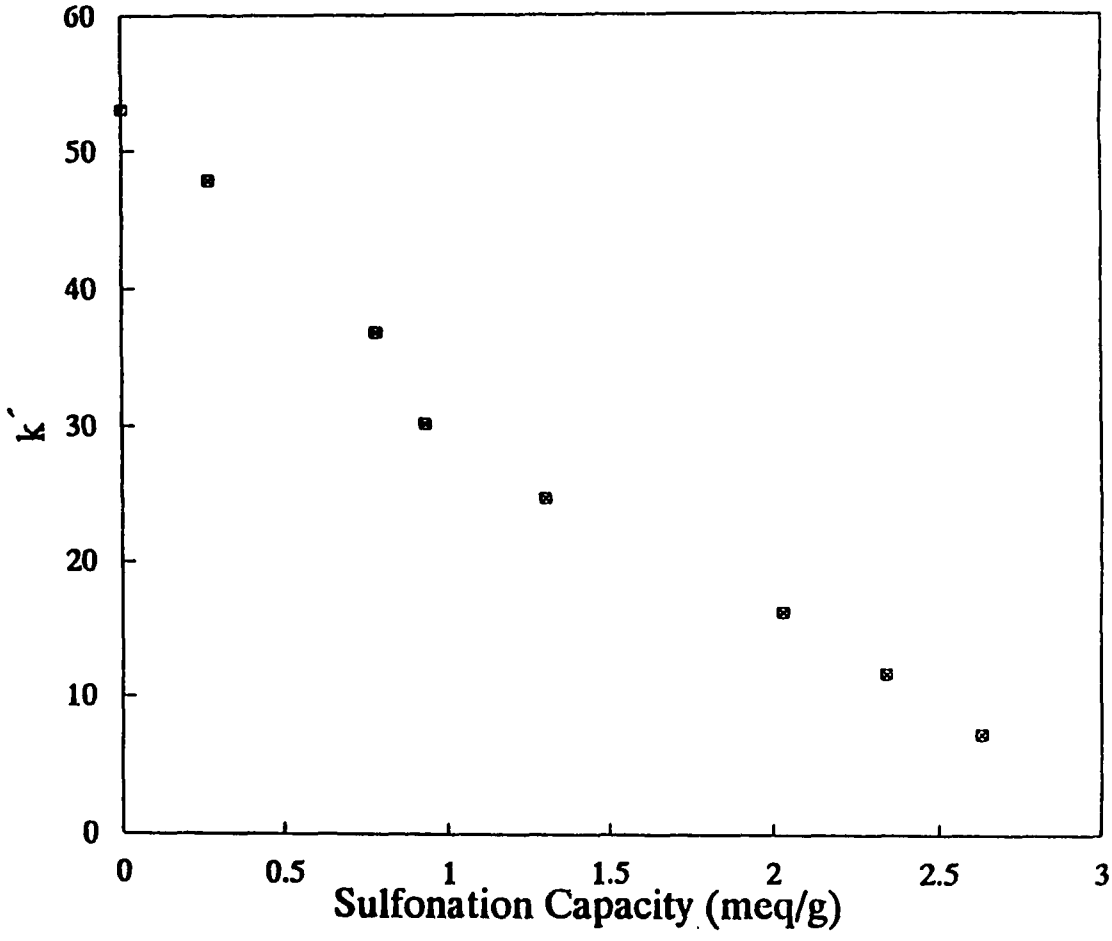

Fig. 1. *k' versus* column sulfonation capacity (milliequivalents per gram) for benzene in 30% acetonitrile, 70% water mobile phase. UV absorbance at 254 nm. Flow rate 1.2 mL/min. 10 cm x 4.6 mm stainless steel columns, 10 μm d_p sulfonated PS-DVB packing. Columns thermostated at 26^0 C.

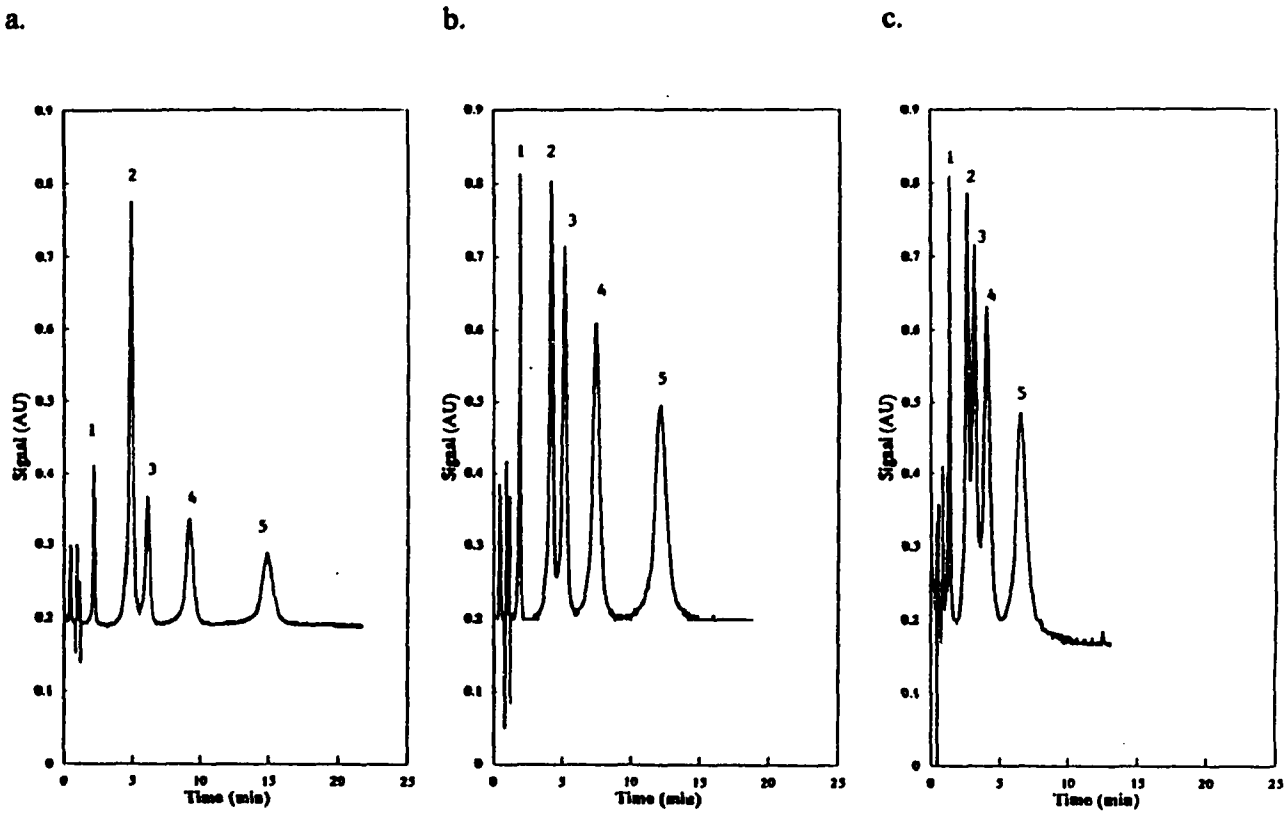

Fig. 2. Chromatograms of 1. *p*-cresol, 2. nitrobenzene, 3. benzene, 4. toluene, and 4. bromobenzene in 50:50 acetonitrile:water on a PS-DVB column sulfonated at a. 0.78 meq/g, b. 0.92 meq/g, and c. 2.03 meq/g. UV absorbance at 254 nm. Flow rate 1.2 mL/min. Column temperature held at 26^0 C.

Table 2
Retention *versus* PS-DVB sulfonation capacity (milliequivalents per gram) for various compounds in 30% acetonitrile, 70% water (retention times taken from the average of at least three injections)

Solute	Retention Time (min)	S.D.	*k'*	Sulfonation Capacity (meq/g)
p-Cresol	7.13	0.05	11.5	0.00
	7.09	0.02	11.4	0.27
	5.54	0.01	8.72	0.78
	4.49	0.01	6.88	0.93
	3.73	0.03	5.54	1.30
	2.72	0.01	3.77	2.03
	2.13	0.01	2.74	2.34
	1.17	0.01	1.83	2.63
Nitrobenzene	26.6	0.3	45.7	0.00
	24.2	0.01	41.5	0.27
	18.6	0.1	31.6	0.78
	15.4	0.1	25.9	0.93
	12.7	0.2	21.3	1.30
	8.61	0.01	14.1	2.03
	6.37	0.02	10.2	2.34
	4.22	0.02	6.40	2.63
Benzene	30.8	0.3	53.0	0.00
	27.8	0.1	47.8	0.27
	21.6	0.1	36.9	0.78
	17.7	0.03	30.2	0.93
	14.6	0.2	24.6	1.30
	9.83	0.07	16.2	2.03

	7.18	0.06	11.6	2.34
	4.66	0.03	7.18	2.63
Toluene	65.6	0.8	114.1	0.00
	58.7	0.4	101.9	0.27
	44.2	0.2	76.5	0.78
	35.4	0.1	61.1	0.93
	28.1	0.4	48.3	1.30
	17.5	0.05	29.7	2.03
	11.9	0.03	19.9	2.34
	6.91	0.04	11.1	2.63
Bromobenzene	-[a]	-[a]	-[a]	0.00
	106	1	184.1	0.27
	84.5	0.7	147.2	0.78
	69.3	0.4	120.6	0.93
	56.3	0.9	97.8	1.30
	35.3	0.1	60.9	2.03
	23.9	0.1	40.9	2.34
	14.0	0.1	23.6	2.63

[a] Prohibitively high retention

authors attributed lower retention to lower overall hydrophobicity due to the higher concentration of sulfonic acid groups. The attraction of water for the hydroxyl group of the polar solute keeps it in the mobile phase.

In the present work, the mobile phase contains a significant amount of organic solvent. This provides wetting of the surface and consequent analyte partitioning into the stationary phase. The observation that both polar and non-polar compounds are retained again suggests that a mixed-mode retention mechanism is at work. Non-polar compounds such as benzene are retained most likely through hydrophobic and π-π interactions between benzene and the aromatic rings of the DVB cross links in the copolymer. The retention of benzene in 30% acetonitrile-70% water in Fig. 1 is an example. Polar solutes such as *p*-cresol and nitrobenzene are also retained under identical conditions but have lower capacity factors than the non-polar solutes (Fig. 2). However, when the mobile phase is changed to contain more organic solvent than water, for example 95:5 acetonitrile:water, polar analytes such as ethylene glycol are retained more by hydrogen bond interactions than by hydrophobic interactions. This is shown in Fig. 3 where retention of ethylene glycol increases with an increase in sulfonation capacity. This increase is progressive under these conditions because the surface of the stationary phase is at all times wetted by acetonitrile. Analytes are thus carried to the stationary phase surface where the hydrogen-bond driven retention takes place. At increasing surface sulfonation, the stationary phase becomes much more polar allowing polar solutes to partition more readily into the stationary phase.

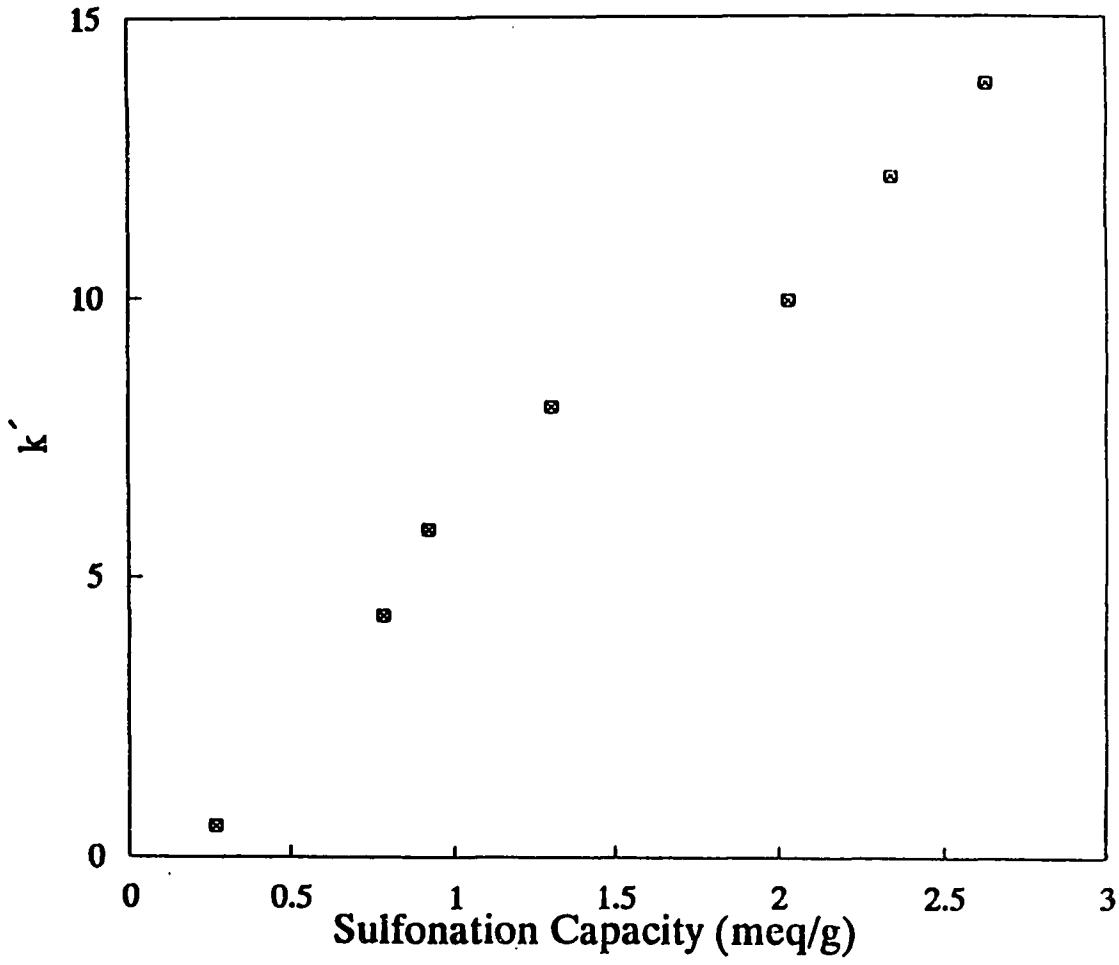

Fig. 3. *k' versus* column sulfonation capacity (milliequivalents per gram) for ethylene glycol in 95% acetonitrile, 5% water. UV absorbance at 195 nm. Flow rate 1.2 mL/min. Column temperature held at 26^0 C.

Effect of column sulfonation on the retention of polar compounds in a normal phase mode

Silica-gel and alumina were used as packing material for early normal-phase separations (liquid-solid chromatography). These materials had strictly polar surfaces. The development of bonded-phase silicas provided columns that had both polar and hydrophobic areas on the surface. These packings have proven to be the most popular as materials for normal-phase separations. The use of sulfonated polystyrene-divinylbenzene columns in normal-phase applications has received little attention in the recent literature. Recently, separations have been shown for polar compounds such as sugars and alcohols in 80:20 acetonitrile:water and under gradient conditions using 0 to 45% water in acetonitrile. These separations were performed on 25 cm Hamilton PRP-X400 columns (sulfonated PS-DVB). Several sugars were separated to near baseline resolution in roughly 15 minutes [18].

In the present work, the same set of sulfonated columns used for the reversed-phase separations in Fig. 1 was used to investigate the retention of polar solutes in a predominantly organic mobile phase. In this case, 95% acetonitrile : 5% water was used as the eluent. With the column having polar groups on the surface of the stationary phase particles and an eluent of high organic content, the system mimics conventional normal-phase chromatography. The advantage in this case, however, is that the number of polar groups on the surface is controlled. A plot of k' *versus* sulfonation capacity is given in Fig. 3 for ethylene glycol. Again the change in retention as a function of sulfonation capacity is approximately linear. The graph has the expected opposite slope compared to benzene in 30:70 acetonitrile:water (Fig. 1), denoting *increased* retention as sulfonation capacity is increased. It was noted in related experiments that polar compounds such as ethylene glycol and propylene glycol were

hardly retained at all when the mobile phase contained a high percentage of water, indicating that hydrophobic interactions between the carbon atoms of the glycols and the polystyrene-divinylbenzene surface are not strong enough to significantly influence retention under these conditions. However, the presence of the sulfonic acid group provides a site for hydrogen-bonding with the hydroxyls of the diols, thus increasing retention for these solutes. Values for capacity factor as a function of sulfonation capacity for a set of polar compounds are listed in Table 3. The best separations were obtained when the column was flushed with 500 mL of 10 mM calcium nitrate before separations were undertaken. An example is shown in Table 4 for three polar compounds on the same 2.34 meq/g sulfonated PS-DVB column in both the hydrogen and calcium forms. All data for Figures 3-7 were collected on columns in the calcium form.

This system can be very useful for the separation of glycols and sugars. An example is shown in the chromatogram in Figure 4 for the separation of three hydroxylated compounds in 95% acetonitrile : 5% water on a column of 0.78 meq/g sulfonation capacity. A sulfonation capacity of 0.78 meq/g or another intermediate sulfonation capacity are good choices for separating this set of solutes in 95:5 acetonitrile:water. High sulfonation capacities in this case would cause analysis times to be too high (note the capacity factor for dextrose in Fig. 4). A high sulfonation capacity could be used to resolve small polar solutes. For compounds such as dextrose and larger, it may be advantageous to use a column of lower capacity.

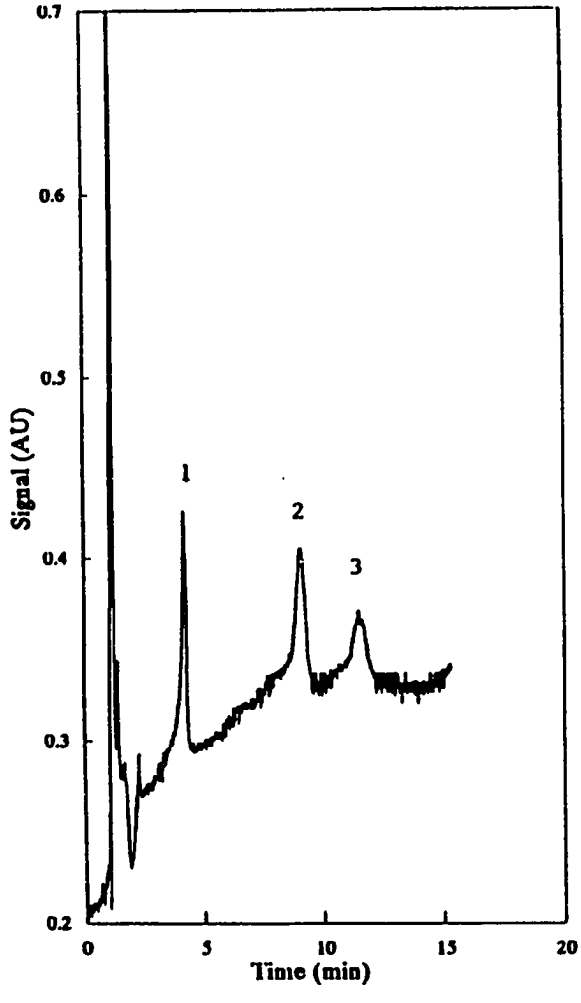

Fig. 4. Separation of 1. propylene glycol, 2. glycerol. and 3. dextrose in 95% acetonitrile, 5% water. PS-DVB column sulfonated at 0.78 meq/g. UV absorbance at 195 nm. Flow rate 1.2 mL/min. Column temperature held at 26^0 C.

Table 3
Retention *versus* PS-DVB sulfonation capacity (milliequivalents per gram) for various compounds in 95% acetonitrile, 5% water (retention times taken from the average of at least three injections)

Solute	Retention Time	S.D.	*k'*	Sulfonation Capacity (meq/g)
Ethylene Glycol	1.14	0.00	0.56	0.27
	3.88	0.02	4.32	0.78
	5.00	0.00	5.85	0.93
	6.61	0.02	8.05	1.30
	7.99	0.01	9.95	2.03
	9.57	0.05	12.11	2.34
	10.8	0.2	13.79	2.63
Propylene Glycol	1.20	0.01	0.65	0.27
	4.15	0.01	4.69	0.78
	5.22	0.02	6.15	0.93
	6.56	0.03	7.99	1.30
	7.45	0.01	9.21	2.03
	8.3	0.1	10.42	2.34
Glycerol	1.43	0.01	0.96	0.27
	6.14	0.01	7.41	0.93
	17.5	0.1	22.92	1.30
	22.1	0.5	29.27	2.03
	28.3	0.5	37.77	2.34

Table 4
Comparison of capacity factors for columns in the hydrogen and calcium forms

Compound	Hydrogen form	Calcium form
Ethylene Glycol	3.13	12.67
Propylene Glycol	2.49	10.83
Glycerol	5.40	35.57

Retention as a function of mobile phase composition

There has been much discussion in the literature regarding the use of log k' as a function of the volume fraction of strong organic solvent (φ) to correlate chromatographic data [19-26]. Many of these discussions have shown plots of log k' *versus* φ where retention is seen to increase with an increase in φ. Such plots have negative slopes when the column is packed with reversed-phase material and when φ represents the fraction of the stronger solvent. The opposite slope would be expected, however, for polar solutes on a sulfonated polystyrene-divinylbenzene column in the presence of an eluent of very high organic content. This is just what was observed when we plotted log k' *versus* φ, when φ represented the fraction of acetonitrile in an acetonitrile/water mobile-phase mixture. Figure 5 shows plots of log k' *versus* φ for five polar compounds in acetonitrile/water eluent on a PS-DVB column of 2.63 meq/g sulfonation capacity. In such a system, it is the water that acts as the stronger solvent. The hydroxyl groups of the polar solutes strongly hydrogen-bond with water, causing greater solute mobility than when the solute is solvated by acetonitrile. Table 5 lists data for log k' *versus* φ where φ is the volume fraction of acetonitrile.

If we now plot log k' *versus* φ, where φ is the fraction of *water* in the binary mixture,

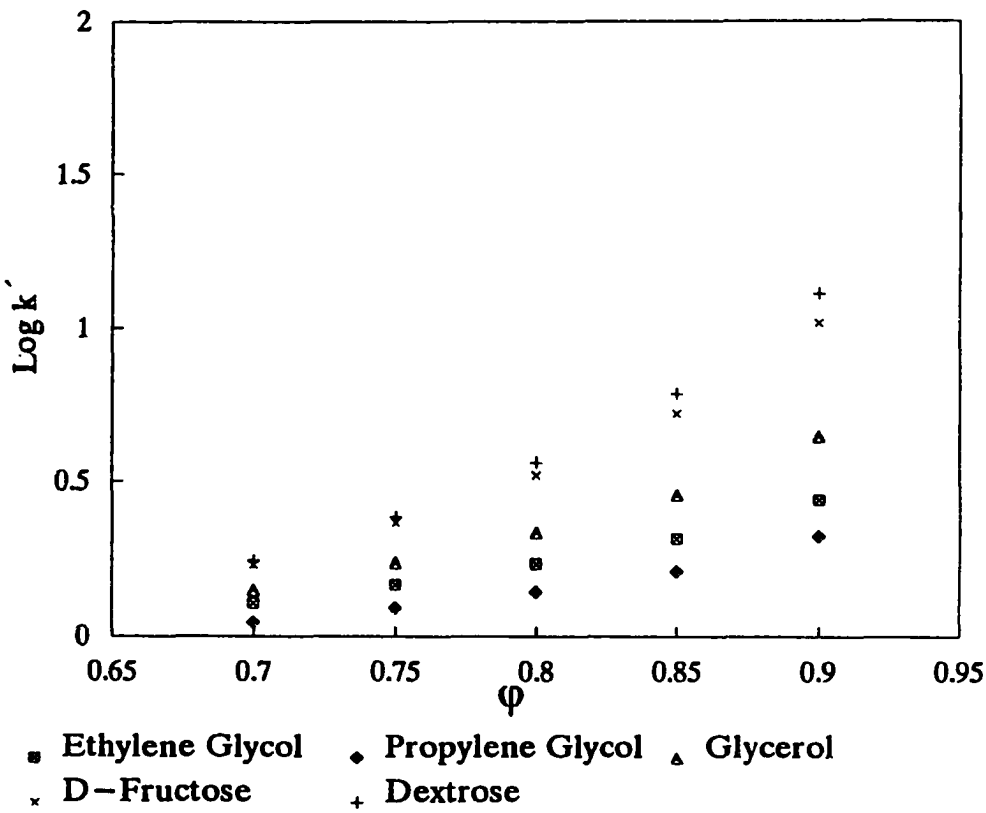

Fig. 5. Graph of log *k' versus* φ (volume fraction of acetonitrile) for ethylene glycol, propylene glycol, glycerol, D-fructose, and dextrose on a PS-DVB column sulfonated at 2.63 meq/g. UV absorbance at 195 nm. Flow rate 1.2 mL/min. Column temperature held at 26^0 C.

we get a graph like that in Fig. 6. In recent papers, we have shown that plots of log k' *versus* φ frequently follow quadratic, rather than linear dependence [2,3]. This trend is again followed for the solutes we tested. An example is shown in Fig. 4.6 for dextrose on a sulfonated PS-DVB column. These data fit a quadratic curve of the form $\log k' = A\varphi^2 + B\varphi + C$ with a correlation coefficient of 0.999 at a 95% confidence level. The values for A, B, and C are 12.0, -9.1, and 1.9, respectively.

A linear correlation can be obtained, however, by using the equilibrium plotting approached presented by us in a recent paper [3]. To adapt this model to the present system, we must let k'_{org} be the capacity factor of a solute in the absence of the stronger solvent. In this case k'_{org} is for the solute dissolved in 100% acetonitrile. If we let R represent the ratio k'_{org} / k', then the following equation, developed in reference 3, can be used

$$\log R = n \log L + \log K \qquad (1)$$

where L is the molar concentration of water, K is the equilibrium constant of the solvation of the solute A by solvent ligand L (water), and n is the slope of the graph representing the combining ratio of L to A. Fig. 7 shows a plot of log R *versus* log L for several polar compounds on the 2.63 meq/g sulfonated PS-DVB column. The data for dextrose show a linear correlation with a coefficient of 0.999. Results for ethylene glycol, propylene glycol, glycerol, and D-fructose are given in Table 6. In this work, the full range of eluent composition was not plotted because the normal-phase effect is lost if the fraction of water is not kept low. Figure 7 shows that the best selectivity lies within the range of log L = 0.9 to 1.2. This corresponds to a range of 15% to 30% water in the acetonitrile/water mobile phase.

Table 5
Log *k' versus* φ where φ = volume fraction of acetonitrile

Compound	Log *k'*	φ
Ethylene Glycol	0.11	0.70
	0.17	0.75
	0.23	0.80
	0.31	0.85
	0.44	0.90
	0.69	0.95
Propylene Glycol	0.05	0.70
	0.09	0.75
	0.14	0.80
	0.21	0.85
	0.32	0.90
	0.57	0.95
Glycerol	0.15	0.70
	0.24	0.75
	0.33	0.80
	0.46	0.85
	0.64	0.90
	0.98	0.95
D-Fructose	0.23	0.70
	0.36	0.75
	0.52	0.80
	0.72	0.85
	1.02	0.90

	1.48	0.95
Dextrose	0.24	0.70
	0.38	0.75
	0.56	0.80
	0.78	0.85
	1.11	0.90
	-[a]	0.95

[a]Not measured

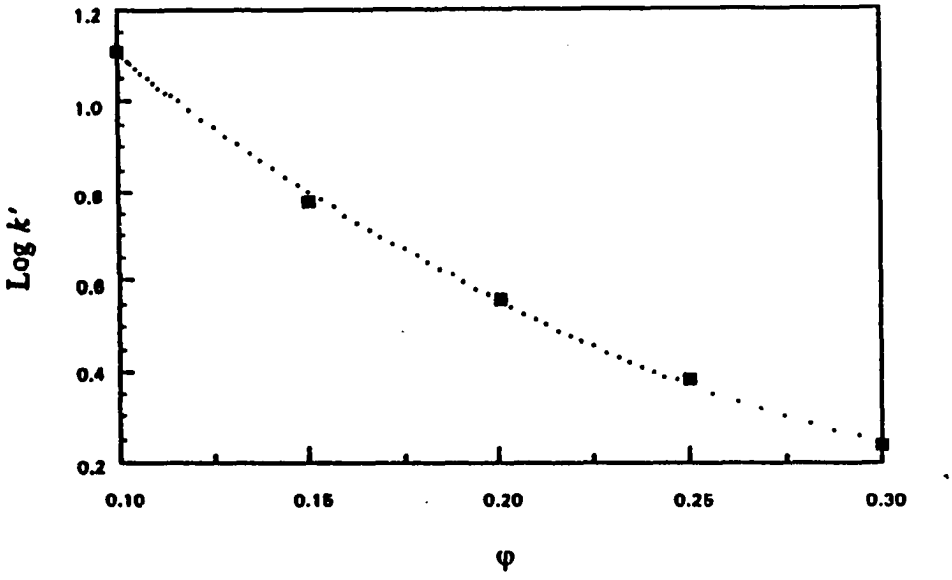

Fig. 6. Graph of log *k' versus* φ (volume fraction of water) for dextrose on a PS-DVB column sulfonated at 2.63 meq/g. UV absorbance at 195 nm. Flow rate 1.2 mL/min. Column held at 26^0 C. Quadratic fit correlation coefficient = 0.999; A = 12.1, B = -9.1, C = 1.9.

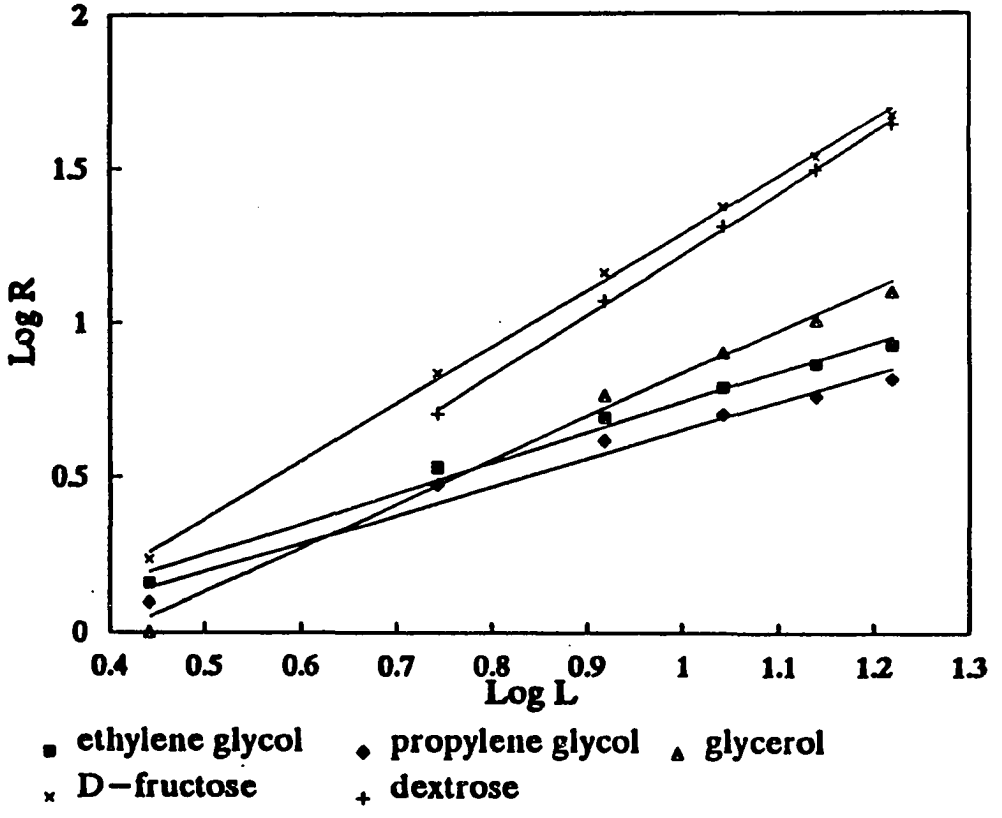

Fig. 7. Plot of log *R versus* log *L* (where *L* is the molar concentration of the strong solvent ligand, in this case water) for ethylene glycol, propylene glycol, glycerol, D-fructose, and dextrose on a PS-DVB column sulfonated at 2.63 meq/g. UV absorbance at 195 nm. Flow rate 1.2 ml/min. Column temperature held at 26^0 C.

Table 6
Correlation results for log *R versus* log *L* for various polar compounds in 95% acetonitrile, 5% water on a PS-DVB column sulfonated at 2.63 meq/g.

Solute	Slope, *n*	S.D.	Correlation Coefficient
Ethylene Glycol	0.97	0.05	0.988
Propylene Glycol	0.90	0.07	0.974
Glycerol	1.39	0.07	0.989
D-Fructose	1.84	0.04	0.998
Dextrose	1.97	0.03	0.999

Conclusions

We suggested in a recent paper [2] that a polar functional group on the surface of a polystyrene-divinylbenzene stationary phase might influence the retention of polar compounds in the presence of traditional normal-phase eluents. It has been shown in the present work that a PS-DVB packing functionalized with sulfonic acid groups can be used to separate hydroxylated compounds in a mobile phase of high organic content. The same column can be used in the presence of reversed-phase eluents for the separation of polar compounds as well as nonpolars like benzene and toluene. A sulfonated PS-DVB column has a great advantage over a silica-based column in that the hydrophilicity of the packing can be controlled. Silicon-oxygen bonds can exist in many forms on a raw silica. Even for reversed-phase silicas the number of residual silanols may vary from column to column. In the preparation of a silica C18 column, steric hinderance precludes the elimination of all the Si-OH groups from the surface of a particle. With a PS-DVB-based material, column preparation begins with a relatively homogeneous hydrophobic surface upon which polar

groups such as SO_3^- are bonded. With careful derivatization, the desired surface coverage of SO_3^- groups can be achieved.

Although the efficiency of PS-DVB columns does not approach that of bonded-phase silicas, they have shown to be useful when separations are undertaken in extreme pH conditions. It can be seen from the results presented here that the same column packed with sulfonated PS-DVB can be used in the reversed-phase and normal-phase modes with the advantage of pH stability.

Such columns could be useful for the separation of sugars in food products. Food material that is extracted with an organic solvent could possibly be separated in the organic extraction matrix on a sulfonated PS-DVB column.

Acknowledgements

The authors thank Doug Gjerde of Sarasep, Inc., for the polystyrene-divinylbenzene material used in this research. Thanks also to Dan Lee of Hamilton Company for information regarding PRP-X400 PS-DVB material. This work was supported by the Office of Basic Energy Sciences, U.S. Department of Energy, and in part by a grant from 3M (St. Paul, MN, USA). We also thank Alltech Associates for the generous gift of the Model 425 HPLC pump used in these experiments. This work was performed at Iowa State University. Ames Laboratory is operated for the U.S. Department of Energy under Contract No. W-7405-Eng-82.

References

[1] T.K. Chambers and J.S. Fritz, *J. Chromatogr. A*, in press.

[2] T.K. Chambers and J.S. Fritz, " High-Performance Liquid Chromatography with Nonaqueous Binary Solvent Mixtures," submitted to J. Chromatogr.

[3] T.K. Chambers and J.S. Fritz, " An Equilibrium-Based Method for Studying the Effect of Solvent Composition on Solute Capacity Factor," submitted to J. Chromatogr.

[4] J. Morris and J.S. Fritz, *Anal. Chem.*, 66 (1994) 2390.

[5] J. Morris and J.S. Fritz, *LC·GC*, 11(7) (1993) 513.

[6] P. Dumont and J.S. Fritz, *J. Chromatogr.*, 691 (1995) 123.

[7] L.L. Lloyd and F.P. Warner, *J. Chromatogr.*, 512 (1990) 365.

[8] L.L. Lloyd, *J. Chromatogr.*, 544 (1991) 201.

[9] J.J. Sun, Thesis, Iowa State University, Ames, IA, 1991.

[10] D.T. Gjerde and J.S. Fritz, *Ion Chromatography*, 2nd edition, Hüthig, Heidelberg, 1987.

[11] G.J. Sevenich and J.S. Fritz, *J. Chromatogr.*, 347 (1985) 147.

[12] G.J. Sevenich and J.S. Fritz, *J. Chromatogr.*, 371 (1986) 361.

[13] G.J. Sevenich and J.S. Fritz, *Reactive Polymers*, 4 (1986) 195.

[14] L. Schmidt and J.S. Fritz, *J. Chromatogr.*, 640 (1993) 145.

[15] P.J. Dumont and J.S. Fritz, *J. Chromatogr. A*, 705 (1995) 149.

[16] P.J. Dumont, Thesis, Iowa State University, Ames, IA, 1995.

[17] P.J. Dumont, J.S. Fritz, and L.W. Schmidt, *J. Chromatogr. A*, 706 (1995) 109.

[18] *Hamilton HPLC Application Handbook*, Hamilton Company, (1993) pp. 85-86.

[19] L.R. Snyder, J.W. Dolan, and J.R. Gant, *J. Chromatogr.*, 165 (1979) 3.

[20] J.W. Dolan, J.R. Gant, and L.R. Snyder, *J. Chromatogr.*, 165 (1979) 31.

[21] K. Karch, I. Sebastian, I. Halász, and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.

[22] R. Tijssen, H.A.H. Billiet, and P.J. Schoenmakers, *J. Chromatogr.*, 122 (1976) 185.

[23] P.J. Schoenmakers, H.A.H. Billiet, R. Tijssen, and L. de Galan, *J. Chromatogr.*, 149 (1978) 519.

[24] P.J. Schoenmakers, H.A.H. Billiet, and L. de Galan, *J. Chromatogr.*, 185 (1979) 179.

[25] L.C. Tan and P.W. Carr, *J. Chromatogr. A*, 656 (1993) 521.

[26] W.J. Cheong and P.W. Carr, *Anal. Chem.*, 61 (1989) 1524.

GENERAL CONCLUSION

This study has focused on two fundamental aspects of high-performance liquid chromatography: the mobile phase and the stationary phase. Working with non-aqueous solvents has provided pertinent information regarding the ability of certain solvents to elute compounds along the chromatographic column based on solute size and structure. This is useful for understanding the retention behavior of a solute in a given solvent alone, without regard for the effect of water. Such information is useful when optimizing HPLC or solid-phase extraction methods. When results from non-aqueous chromatographic experiments are compared with those of aqueous/organic chromatography, a broader understanding of the retention mechanism follows. For example, this work has provided chromatographic support for the hypothesis that a methanol:water associated complex exists in a binary eluent.

Silica-based columns offer many advantages to the chromatographer, including high column efficiency and pressure stability. These columns, however, can be degraded when mobile phases of very low or very high pH are used, resulting in the loss of reproducibility and efficiency. The surface of a bonded silica particle also contains many forms of hydroxyl groups, which may affect retention and reproducibility of peaks.

Polystyrene-divinylbenzene columns are stable under extreme pH and are a worthy alternative to bonded silica columns. In this work, the use of polystyrene-divinylbenzene as a stationary phase has allowed measurements that can be difficult or impossible on silica-based material. The hydrophilicity of PS-DVB particles can be controlled by careful surface derivatization with sulfonic acid groups, resulting in greater peak reproducibility and no interference from silica-based hydroxyl groups. The controlled hydrophilicity of the

sulfonated PS-DVB columns provides the opportunity for greater analyte selectivity.

Separate columns are usually used for reversed-phase and normal-phase separations. Columns used in this work were used in both reversed-phase and normal-phase modes, which can be useful for separations of compounds dissolved in aqueous and non-aqueous matrices, such as extracted material.

This work has also shown a simple method for using chromatographic measurements to correlate retention phenomena. In this way, one can avoid spectrometric or other types of measurements that are often required for other retention models. The model presented here will be useful for method optimization and may be useful as a predictive tool in solid-phase extraction and high-performance liquid chromatography.

ACKNOWLEDGEMENTS

I would like to extend thanks to Dr. James S. Fritz for his expert advice and guidance during the course of this study. He is highly regarded in the field of analytical separations and I consider it an honor to have worked under his direction.

The members of my Program of Study Committee have also provided excellent guidance and advice, for which I am grateful: Dr. R.S. Houk, Dr. Therese Cotton, Dr. George Kraus, and Dr. C. Oulman. Thanks to Dr. Ed Yeung for sitting as a substitute on my POS committee and for providing work during the summer of 1991, before I began as a graduate assistant. Thanks to Dr. Ali Tabatabai for joining my committee at this late date. Thanks also to Dr. Dennis Johnson for support and encouragement.

I thank my wife, Sarah, for continual support and encouragement during my time as a graduate student. I'm also grateful to my parents, sisters, and in-laws for moral and financial support when things got tough. Thanks to Dr. Charles Hanson for encouraging me to seek a professional degree and for believing that I could do it. Many thanks also to Dr. Robert Eierman, Dr. Fred King, Dr. J. Roger Selin, Dr. Walter Wussow, Mr. Gerald Jahn, Dr. Jerome Mullin, and Dr. Beverly Pestel for guiding me along the path to a career in chemistry. I thank Rev. Gary Pilcher for his spiritual guidance during our five years in Ames. Thanks also to Tony Rivera and Henry Kern for their witness and support.

I am grateful to Supelco, Inc. and to 3M Company for providing my stipend and equipment budget. Raaidah Saari-Nordhaus of Alltech Associates and Doug Gjerde of Sarasep, Inc., are also to be thanked for equipment and materials.

Most importantly, I thank God for turning my life around and also for giving me the

desire and the wherewithal to complete and advanced degree in science.

This work was performed at Ames Laboratory under Contract No. W-7405-Eng-82 with the U.S. Department of Energy. The United States government has assigned the DOE report number IS-1788 to this thesis.